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DETECTION OF THE BIODEGRADABILITY OF CELLULOSIC BIOMASS BY DIELECTRIC PARAMETERS

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Abstract

In biomass utilization technologies enzymatic hydrolysis and fermentation processes are widely used. Development of rapid and non-destructive measurement methods is needed to detect the enzymatic biodegradation of cellulosic biomass, for instance. In our researches the applicability of dielectric measurement methods was investigated for the monitoring the efficiency of enzymatic hydrolysis of Cobex (corn cob) biomass. The dielectric behavior of the Cobex suspensions and fermentation broth was characterized by the dielectric constant and dielectric loss factor, measured at a frequency range from 200 to 2400 MHz. The dielectric parameters were also determined during the ethanol fermentation process of the preliminary hydrolyzed biomass. Our results verified, that the dielectric parameters in the frequency range of 200-100 MHz are sensitive to the chemical changes occurred during the enzymatic hydrolysis of cellulose contented biomass, and, as well as to the presence of ethanol component in the fermentation broth. There was found good correlation of dielectric constant (at frequency range from 300 to 900 MHz) with the concentration of reducing sugars (produced in enzymatic hydrolysis), and the dielectric constant and dielectric loss factor (determined at the frequency of 300 MHz) with the ethanol concentration.

Introduction

The dielectric properties of biomaterials and biosystems have been investigated for decades to make possible the appropriate planning and design of processing equipment operating at microwave and radio frequencies. Dielectric constant (ϵ ') measures the ability of materials for storage of energy in electric field; dielectric loss factor (ϵ '') corresponds with the energy dissipation of materials. Open-ended coaxial line sensors connected to vector network analysers are commonly used for the measurement of the dielectric properties and dielectric behaviour of materials at wide frequency ranges [1].

Dielectric properties are responsible for the materials-electromagnetic field, distribution of electromagnetic field inside of the materials [2]. In high water contented material the dielectric constant decreases with frequency, if polar molecules can follow the polarity change of the electromagnetic field. Nevertheless, over a critical frequency value, phase lag occurs between the dipole rotation and the change of the polarity of electric field. The two main dielectric loss mechanisms can be the dipole rotation and ionic conduction, depending on the applied frequency and the compounds of the system. Considering the frequency range of microwave heating should be noted that ionic conduction plays crucial role in heating efficiency at 915 MHz [3]. But, depending on the temperature and the components of the system, both the dipolar mechanisms and ionic conduction have also effect on the dielectric behaviour and thermal efficiency of microwave irradiation at the frequency of 2450 MHz [4].

In high water contented medium the dielectric parameters of water determined mainly the dielectric behaviour of the system [5]. The temperature has also effect on dielectric behaviour.

In polar dispersion region, the dielectric constant increases as temperature increases, but at other frequency ranges an opposite tendency can be found. In the frequency range of 300-3000 MHz the temperature increment cause the decreasing of dielectric constant, because the higher thermal energy makes difficult for dipolar components to align with the polarity change of electric field [1]. Considering the effects of water content on dielectric parameters, it can be concluded that the bound water contributed less in the dielectric behaviour of the materials, than the free water content. Free water molecules - as polar components - can orient easier in varying polarity electromagnetic field. Increase of the concentration of ionic components led to decrease of dielectric constant and increase of dielectric loss factor, because the dissolved salts are conductors in electric field [6]. The increase of water content in suspensions decrease the viscosity, and, therefore the 'binding' forces playing role in ionic conduction mechanisms are decreased [7].

One of the most important key compounds produced in enzymatic hydrolysis of lignocellulosic biomass is the glucose. Measurement of the glucose concentration enables to monitor the cellulose degradation process due to chemical, thermochemical and enzymatic processes, respectively. In glucose and sucrose contented solution has been verified, that relative permittivity decreased as the concentration increases. But, if salts are present in the solution generalized tendencies cannot be given at the frequencies higher than 200 MHz [4]. Beside the detection of chemical changes, the biological changes of materials can be monitored by dielectric measurements. Zhu et al. [8] verified that bacterial growth increases the capacitance of milk samples that led to increased dielectric constant. If microorganisms can decompose the macromolecules into lower molecular weight products the conductivity of the medium increases, therefore, at radio frequency ranges, the dielectric loss is influenced mainly by ionic conduction. Kouzai et al. [9] developed waveguide penetration method to determine the dielectric behaviour of fermentation broth. They verified, that the decrease of glucose concentration and the increase of ethanol concentration are correlated well with the complex permittivity. Arnoux et al. [10] used the permittivity to monitor the biomass production in lactic acid fermentation process. Olmi et al. [11] applied dielectric measurement method for on-line detection of the efficiency of sugar/alcohol conversion and carbon dioxide production in beer fermentation process.

The main objective of our work was to investigate the applicability of dielectric parameters for the detection of the efficiency of enzymatic hydrolysis and fermentation of cellulosic biomass.

Experimental

Dielectric constant (ϵ ') and dielectric loss factor (ϵ '') are measured at frequency range from 200 to 2400 MHz by open-ended coaxial-line probe (SPEAG DAK 3.5), connected to a vector network analyzer (ZVL-3 VNA, Rhode&Schwarz). Samples were measured in polytetrafluoroethylene (PTFE) tube container (diameter of 30 mm, volume of 35 mL). Immersion depth of DAK probe was 10 mm, temperature of samples was controlled at 25°C by water bath. The averaged dielectric parameters were calculated from 30 measuring points.

Corn cob residues (COBEX F12/30) was used as raw material for the enzymatic hydrolysis tests, which has an average particle size of 840 μ m, moisture content of 7.3 w%, and cellulose and hemicellulose content of 32.1% and 37.3%, respectively. For the 7 days enzymatic hydrolysis tests Cellic CTEC2 (Novozymes) industrial enzyme blend (with cellulase, β -glucosidase and hemicellulose activities) was used at the temperature of 40°C and pH of 4.8 using 3.5 w% TS contented Cobex suspensions. For the ethanolic fermentation stage commercial *Saccharomyces cerevisie* was applied in 0.5 w% concentration. For the enzymatic hydrolysis and fermentation experiments Labfors Minifors (Infors) bioreactors were used.

The cellulose degradation was characterized by the concentration of reducing sugars measured by DNSA method. Ethanol concentration of the fermentation broth was measured based on refraction index, after distillation. Beside the non treated Cobex samples (Cont.), alkaline (Alk.) pre-treatment (dosage of 50 mg/g_{TS} NaOH) and microwave (MW) pretreatment (100 mL of suspension with MW power of 500 W for 4 minutes at 2450 MHz frequency) were also applied before the enzymatic hydrolysis to increase the biodegradability of the biomass.

Results and discussion

In the first series of our experiments, the dielectric behavior of non-treated (Cont.) alkaline (Alk.) and microwave (MW) pretreated biomass was investigated as the change of dielectric constant as the function of measuring frequencies. Our results show, that partial decomposing of cellulose fraction due to microwave pre-treatment resulted in the decreased dielectric constant, compare to the control sample. However, the dosage of NaOH increased the dielectric constant of Cobex suspension (Figure 1.). This increment is caused mainly by the strengthening of ionic conduction mechanisms [4]. For a given sample, the dielectric constant decreases as the hydrolysis time increased. These establishments are in a good agreement with the literature: in the biomass pre-treatment step, and/or the enzymatic hydrolysis process the macromolecules decompose to smaller molecular weight components, which can be polarized easier in the electric field [9]. This effect can be manifested in the decrease of dielectric constant.



Figure 1. Dielectric constant of enzimatically hydrolized samples in the frequency range of 200-2400 MHz (t=25°C) (,d' denotes the days of enzymatic hidrolysis)

Our research aimed the investigation of the relationship between the dielectric parameteres and the degree of celluose hydrolysis. The cellulose degradation was characterized by the change of the concentration of reducing sugars (RS). The experimental results show a good correlation of dielectric constant at lower frequencies with the concentration of reducing sugars. At frequencies of 300 MHz and 900 MHz the relationship can be given by quadratic or linear equation for alkaline pre-treated (Alk.), or control (Cont.) sample, respectively (Figure 2.).



Figure 2. Correlation between dielectric constant and the concentration of reducing sugars

Olmi et al. [11] found a good correlation between the dielectric loss factor and ethanol concentration in a four steps beer fermentation process. Therefore, measurements were conducted to examine the dielectric parameters during the ethanol fermentation of hydrolized corn cob biomass.

It was verified that the sugar/ethanol conversion (production of ethanol from sugary by yeast fermentation) can be detected by the dielectric parameters. At the measuring frequency of 300 MHz a good correlation was found between the ethanol concentration and dielectric constant and dielectric loss factor, as well (Figure 3.).



Figure 3.Change of dielectric constant and dielectric loss factor as a function of ethanol concentration (f=300 MHz, t=25°C)

Conclusion

The main aim of our research was to investigate the relationship between the dielectric parameters and conventionally used analytical parameters in the biological utilization of cellulosic biomass.

Our results show that the reducing sugar yield in the enzymatic hydrolysis process was in a good correlation with the dielectric constant determined at lower frequency range.

In fermentation process, the sugar/ethanol conversion rate can be detected by the measurement of dielectric constant and dielectric loss factor, as well.

These results verified the applicability and usability of dielectric measurements, as a nondestructive, chemical-free and rapid method, for the monitoring of enzymatic hydrolysis of cellulosic biomass and ethanol fermentation process, as well.

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METHANOL OXIDATION CATALYST BY ATOMIC LAYER DEPOSITION

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Abstract

Direct liquid fuel cells (DMFCs) are very appealing alternatives for fighting climate change, particularly in the field of personal mobility solutions. However, DMFCs also have some serious competitive disadvantages, like the high cost of the noble metal catalysts, the difficulties of the catalyst application, and the poisoning of the catalyst due to carbon monoxide formation. Here we demonstrate that depositing platinum on TiO₂ by atomic layer deposition (ALD) is an easy, reproducible method for the synthesis of TiO₂-supported platinum catalyst for methanol oxidation with excelent anti CO poisoning properties.

Introduction

Direct liquid fuel cells (DMFCs) enjoy increasing scientific attention today due to their low working temperature, high power density, and compact size. The most universally used catalysts in DLFCs today are platinum and platinum group metals. Despite of its drawbacks, platinum shows excellent electrocatalytic properties in most fuel cells. It is the most used catalyst for both the cathodic side as oxygen reduction reaction (ORR) and the anodic side as hydrogen oxidation reaction (HOR) or methanol oxidation reaction (MOR) catalyst. However, during the last process, carbon monoxide forms as an intermediate that can have serious negative effects on the performance of the catalyst due to the strong CO-Pt interactions. Transition metal oxides, including titanium oxides, are widely used as catalyst support materials due to their high surface area and excellent chemical stability. In MOR metal oxides also help the oxidation of carbon monoxide and reduce anode catalyst poisoning. One of the main setbacks of the oxides is their low electrical conductivity, especially compared to the other supporting materials used in fuel cells, such as carbon structures or metal foams [1],[2],[3],[4]

Experimental

Working electrodes were synthesised by atomic layer deposition, depositing a titanium dioxide layer and platinum nanoparticles on AvCarb P75 carbon paper support. The Beneq TFS 200 ALD equipment was used during the process. To prepare the electrode, first 25 cycles of TiO₂, then 20 cycles of platinum were deposited on the same GDL (25c TiO₂ & 20c Pt). For comparison, we also prepared carbon paper/Pt nanoparticle electrodes without the underlying titanium dioxide layer (20c Pt). Before and after the synthesis the weight of the carbon paper was measured to calculate the weight of the deposited materials. The TiO₂ loading was 0.019 mg cm⁻², while the platinum loadings were 0.277 and 0.116 mg cm⁻² in 20c Pt and 20c Pt & 25c TiO₂ catalysts, respectively.

The as-synthesized catalysts were characterized by transmission electron microscopy. A FEI Tecnai G2 20 X-Twin microscope was used. The crystal structure of the electrodes was analyzed by powder X-ray diffraction measurements (XRD). The Cu K α radiation was generated by a Philips PW1830 X-ray generator operating at 40 kV.

The electrochemical measurements were conducted in a custom made three-electrode PTFE cell using an ACM Instruments Gill AC potentiostat at room temperature. ALD modified AvCarb P75 carbon papers were used as working electrodes, while Ag/AgCl (3 M NaCl) and Pt wire were utilized as reference and counter electrodes, respectively. The two halves of the cell were separated by a glass frit. During the measurements 0.5 M sulphuric acid and 0.5 M sulphuric acid + 0.5 M methanol were used as electrolytes. The electrochemical properties of the ALD modified electrodes were examined by cyclic voltammetry between 0 and 1200 mV vs RHE at a sweep rate of 10 mV s-1. The electrochemical surface area (ECSA) of the catalysts was calculated from the hydrogen adsorption/desorption region of the obtained voltammograms by the following equation:

$$ECSA\ (m^2 g_{Pt}^{-1}) = \frac{Qh\ (mCcm^{-2})}{0.21(mC\ cm^{-2}) * W_{Pt}(mg_{Pt}cm^{-2})} * 10^{-1}$$

Here, Qh is the charge calculated from the H_{des} region of the voltammogram, while the 0.21 represents a charge required to oxidize a monolayer of hydrogen adsorbed on Pt and the W_{Pt} is the loading of platinum [5],[6]. The methanol oxidising properties and the CO tolerance of the electrode were evaluated from the CV measured in the methanol containing electrolyte by comparing the ratio of the peak current during the forward (i_f) and the backward (i_b) scan. It is generally accepted that the ratio of these peaks is correlated with the tolerance of the catalyst to carbonaceous species. An increase in the i_f/i_b ratio means enhanced CO oxidation properties of the catalyst [7].

Results and discussion

As can be seen in Fig 1.(a,b) platinum nanoparticles are well distributed on the surface of the carbon support, while only fewer nanoparticles formed in the titanium dioxide containing gas diffusion electrode. The average particle sizes were 3.2 nm, and 2.4 nm in 20c Pt and 25c TiO₂ & 20c Pt catalysts, respectively. The difference in particle size could be attributed to the difference of the surface energy, and consequently, the wettability of the carbon and TiO₂ covered carbon supports. The smaller particle size implies higher surface area, which in turn means more active sites for methanol oxidation.



Figure 1.: TEM images(a,b) and the characteristic XRD patterns of the ALD synthesised 20c Pt and 25c TiO2 & 20c Pt catalysts

Fig 1.(c) shows the XRD patterns of the ALD-synthesised electrodes. In the case of 20c Pt electrode, the two reflections at 39.5° and 44.4° correspond to the platinum (111) and (200) planes. In the case of the titanate containing electrode, the reflections of platinum are broadened

because of the smaller particle size. In bare carbon paper, the two reflections are attributed to the (100) and (101) planes of the graphite content of the carbon fibre GDL.

The electrocatalytic properties of the ALD-synthesised electrodes were investigated by cyclic voltammetry (CV). The hydrogen adsorption/desorption region is visible between 0 and 0.4 V (vs. RHE) in Fig 2. (a).



Figure 2.: The cyclic voltammograms measured in 0.5 M H_2SO_4 (a) and 0.5 M methanol / 0.5 M H_2SO_4 electrolytes (b), the elec-trochemically active surface area (c) and the mass activity (d) of the ALD synthesised catalysts compared to the drop-casted platinum nanoparticle catalysts

The electrochemical surface area was calculated as 55.35 and 80.05 m2 gPt⁻¹ for the 20c Pt and 25c TiO₂ + 20c Pt catalysts, respectively. The methanol oxidation properties of the electrodes were evaluated by measuring cyclic voltammograms in 0.5 M sulphuric acid/0.5 M methanol electrolyte. The higher the i_f/i_b ratio (i.e., the ratio of the peak current of the forward (i_f) and backward (i_b) scan), the more efficient the oxidation of methanol to CO₂, which means less carbonaceous species accumulation on the surface of the catalyst [8].

Conclusion

Atomic layer deposition is a suitable method to synthesize well-distributed platinum nanoparticles on carbon paper and titanate covered carbon paper supports, where both the platinum and TiO_2 were synthesised by ALD. The morphology and the crystal structure of the as-prepared electrodes were characterised and the electrocatalytic methanol oxidation activity was evaluated. This yielded platinum nanoparticles with high electrochemical surface area and mass activity with the use of titanate covered carbon paper GDL support. The mass activity of the new catalyst exceeded that of the reference Pt catalyst obtained by traditional wet chemistry

by one order of magnitude. Moreover, the increased i_f/i_b ratio leads to better methanol oxidation properties and renders the reported preparation method a promising alternative for creating poisoning tolerant anode catalysts in DMFCs.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF PHARMACEUTICALLY RELEVANT ISOPULEGOL-BASED COMPOUNDS

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The chiral separation of biologically active compounds, in the case of the synthesis of enantiomerically pure drugs, is a particularly important application area of HPLC in pharmaceutical analysis. β -Amino lactones and β -amino amides are pharmaceutically important molecules for several reasons. For example, water-soluble derivatives of the β -amino lactones can exhibit cytotoxic activity through a prodrug mechanism for different human cancer cell lines [1]. β -Amino amides are well-known subunits of biologically-important compounds such as bestatin, a potent aminopeptidase B, which is quite useful in the treatment of cancer through its ability to enhance the cytotoxic activity of known antitumor agents [2]

In this study, the separation of enantiomers of isopulegol-based β -amino lactones and β -amino amides was studied on seven covalently immobilized polysaccharide-based chiral stationary phases. The separation of the stereoisomers was optimized by investigating the effects of the composition of the bulk solvent and the influence of the temperature on the chromatographic behavior. Since the enantiorecognition mechanisms of the polysaccharide-based selectors are not entirely known [3], the elution orders of the enantiomers cannot be predicted. Therefore, during our work, close and thoughtful attention was paid to the elution sequences. In addition, the relationships between the compound's structure and the chromatographic parameters were also investigated. Experiments were performed in the temperature range 10–50 °C and thermodynamic parameters were calculated from plots of ln α versus 1/T. The separations were generally enthalpy-controlled, but entropy-controlled separation was also observed. Our results contribute to a better understanding of the enantiorecognition mechanism of polysaccharide-based chiral stationary phases.

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FACTORIAL DESIGN ANALYSIS OF PHOSPHATE REMOVAL FROM MODEL SOLUTION BY IRON-LOADED POMRGRANATE PEEL

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Abstract

This study investigated the removal of phosphate (PO_4^{3-}) from disodium hydrogen phosphate (Na_2HPO_4) solution using Iron-loaded pomegranate peel (IL-PP) as a bio-adsorbent. The full factorial design using Minitab19 software was applied to analyze the effects of influencing parameters and their interactions and derive the equation that adequately describe PO_4^{3-} removal by IL-PP. Effective PO_4^{3-} removal up to 90% was achieved within 60 min, at pH 9 and 25 °C temperature using a 150 mg dose of IL-PP.

Introduction

Excess release of phosphorus is the main culprit for the eutrophication of freshwater and marine ecosystems [1]. Furthermore, phosphorus which is a critical element for plant growth is threaten by exhaustion [2]. Therefore the recovery of phosphate from wastewater is highly required protect the ecosystem and sustain the environment. Biosorption process using agricultural and food waste (AFW) presents several advantages such as simplicity, cost-effectiveness, and wide availability of potential bio-adsorbents that can be further applied to soil as fertilizer after adsorption of phosphate.

Pomegranate peel (PP) is among the widely abundant AFW [3]. Valorization of PP as bioadsorbent for heavy metals, dyes and other contaminants removal was investigated in several studies and it showed promising adsorptive characteristics in both raw and activated forms or even as active carbon [4–6]. The present study investigated the efficiency of IL-PP at PO_4^{3-} removal from Na₂HPO₄ model solution using factorial design methodology in order to prove the performance of IL-PP as an alternative to conventional phosphate adsorbents.

Experimental

 PO_4^{3-} removal rate was calculated as follows:

Removal
$$\% = \frac{C_i - C_f}{C_i}$$
. 100

Where C_i (mg·L⁻¹) and C_f (mg·L⁻¹) are the initial and final PO₄-P concentrations, respectively.

 2^{3} full factorial design was used to evaluate the effect of pH, adsorbent dose, temperature and their interactions on PO₄³⁻ removal by IL-PP. Factorial design plots such as plots for the main effects and interactions, Pareto chart, and normal plot for the standardized effects describe how the effect of one factor varies with the level of the other factors. This technique investigates all possible combinations and verifies the accuracy of the obtained mathematical model through the analysis of variance (ANOVA) to achieve optimum removal of PO₄³⁻. Parameters such as initial PO₄-P concentration (40 mg.L⁻¹), contact time (60 min) and stirring speed (150 rpm) were kept constant and the three factors pH, adsorbent dose, and temperature of solution were varied at two levels as given in (Table 1). A centre point was duplicated and added to matrix in order to verify the curvature of the studied model.

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Table 1. Tactors and levels used in the factorial design for 1.04 Telloval by IL-11								
Factor	Symbol	Low level (-1)	High level (+1)	Centre point $(0, 0, 0)$				
pН	А	3	9	6				
Adsorbent dose	В	100	150	125				
Temperature	С	25	45	35				

Table 1. Factors and levels used in the factorial design for PO_4^{3-} removal by I

Results and discussion

The mean of the experimental results for the respective high and low levels of pH, adsorbent dose and temperature are shown in Figure 1.



Figure 1. Cube plots for PO₄³⁻ removal by IL-PP

Table 2 presents the main effects and interactions, model coefficients, standard deviation of each coefficient, standard errors, Fisher test value (F-value), and probability value (P-value). Table 2. Estimated effects and coefficients for PO_4^{3-} removal by IL-PP

Term	Effect	Coef	SE coef	t-value	p-value	VIF		
Constant		71.195	0.125	569.56	0.001			
pH (A)	12.650	6.325	0.125	50.60	0.013	1.00		
Adsorbent dose (B)	32.565	16.282	0.125	130.26	0.005	1.00		
Temperature (C)	5.440	2.720	0.125	21.76	0.029	1.00		
A*B	-6.855	-3.428	0.125	-27.42	0.023	1.00		
A*C	-1.480	-0.740	0.125	-5.92	0.107	1.00		
B*C	-2.145	-1.072	0.125	-8.58	0.074	1.00		
A*B*C	-1.065	-0.533	0.125	-4.26	0.147	1.00		
Ct Pt		0.055	0.280	0.2	0.876	1.00		
S	0.135015							
R^2	100.00%							
R^2 (Adj)	99.96%							

The effects of pH (A), adsorbent dose (B), temperature (C), and the interaction (A*B) were significant at a 5% probability level (P < 0.05). However the effects of interactions (A*C), (B *C) and (A*B *C) were not significant (P > 0.05). Furthermore, the adjusted square correlation coefficient R² (adj) had a value of 99.96%, which indicates that the presented model perfectly fit the statistical model [7].

In this way, PO_4^{3-} removal by IL-PP could be expressed using Eq. (1).

Phosphate removal% = $71.195 + 6.325 A + 16.282 B + 2.720 C - 3.428 A \cdot B + 0.740 A \cdot C - 1.072 B \cdot C - 0.533 A \cdot B \cdot C + 0.055 Ct Pt$ (1)

where: A (pH), B (adsorbent dose), C (temperature), AB, AC and BC (their 2- way interaction) and ABC (their 3-way interaction). ($3 \le pH \le 9$, 100 mg \le Adsorbent dose ≤ 150 mg, 25 °C \le temperature $\le 45^{\circ}$ C).

ANOVA was performed to investigate the significance of parameters affecting PO_4^{3-} removal to ensure the accuracy of the model. Table 3 presents the sum of the squares used to estimate the effect of factors, the F-ratio (i.e., the ratio of individual mean square effects to the mean square error) and the P-value (i.e., the level of significance leading to the rejection of the null hypothesis). The results showed are in accordance with the estimated effects shown in Table 2 and thus confirm the accuracy of the factorial design model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	2610.03	1118,32	2610.03	0.015
Linear	3	2500.19	2499.60	6667.18	0.009
pH (A)	1	320.05	959.50	2560.36	0.013
Adsorbent dose (B)	1	2120.96	6361.90	16967.67	0.005
Temperature (C)	1	59.19	177.40	473.50	0.029
2-Way Interactions	3	107.56	107.54	286.84	0.043
A*B	1	93.98	281.88	751.86	0.023
A*C	1	4.38	13.13	35.05	0.107
B*C	1	9.20	27.63	73.62	0.074
3-Way Interactions	1	2.27	6.77	18.15	0.147
A*B*C	1	2.27	6.77	18.15	0.147
Curvature	1	0.00	0.00	0.04	0.876
Error	1	0.13	0.02		
Total	9	2610.15			

Table 3. ANOVA for PO₄³⁻ removal by IL-PP

Figure 2 shows the main effects of each parameter on PO_4^{3-} removal by IL-PP and thus helps to identify which parameters affect the response variable the most. A larger deviation is synonymous with a large effect [8]. Accordingly, adsorbent dose appears to have the greater effect on PO_4^{3-} removal by IL-PP, followed by pH and then temperature that has a negligible effect.



Figure 2. Main effects plot for PO₄³⁻ removal by IL-PP

Figure 3 plots the interactions of the studied parameters. If the interaction lines are not parallel, this implies that the interaction has a strong effect, whereas parallel interaction lines indicate a weak effect Interpretation of interaction plot implies that if the interactions lines are not parallel, the interaction under control is strong and vice versa [9]. The most important interaction for PO_4^{3-} removal by IL-PP appears to be (pH × adsorbent dose), followed by (adsorbent dose × temperature). The least important interaction was (pH × temperature), which had almost parallel interaction lines.



Figure 3. Interaction plot for PO₄³⁻ removal by IL-PP

A Pareto chart can be used to evaluate the significance of effects on the basis of how much they exceed the reference line [10]. Figure 4 shows that (A), (B), their interaction (AB), and (C) had a significant effect because their values exceeded that of the reference line (12.7, in red). However, the effects of interactions ($B \times C$), ($A \times C$) and ($A \times B \times C$) are not significant as their values didn't exceed the red line.



Figure 4. Pareto chart of the standardized effects for PO_4^{3-} removal by IL-PP The results of 2^3 factorial design have showed that pH (A), adsorbent dose (B), their interaction (AB), and temperature (C) are the factors with significant effect on the PO_4^{3-} removal from Na₂HPO₄ solution using IL-PP. On the other hand, the effect of temperature between high and low level was very weak and tends to be negligible (0.25%) when using optimum pH (9) and adsorbent dose (150 mg), therefore for technical and cost-effectivity reasons, a reduced model that take in consideration only the significant factors and neglect temperature is suggested. The new model is 2^2 factorial model with two factors (pH and adsorbent dose) at two levels and can be described using Eq. (2):

Phosphate removal% = $71.19 + 6.33 A + 16.28 B - 3.43 A \cdot B + 0.06 Ct Pt$ (2)

Conclusion

The efficiency of IL-PP to remove PO_4^{3-} from aqueous solution was evaluated in this study. Results introduce IL-PP as an efficient bio-adsorbent which could be used in a green technology for wastewater treatment, waste biomass management and phosphate recovery.

Acknowledgements

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PASSIVE SAMPLING FOR PESTICIDES AND PAHs IN THE SIMONA PROJECT

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Abstract

Passive samplers (solid phase extraction, SPE disks and Polar Organic Chemical Integrative Sampler, POCIS) were tested to monitor 85 pesticides (including glyphosate and aminomethylphosphonic acid, AMPA), 19 polycyclicaromatic hydrocarbons (PAH) components, metals in the Drava river. Among pesticides the time weighted average concentrations of terbuthylazine, S-metolachlor and tebuconazole were the highest. Some chlorophenoxy acids (2,4-D, mecoprop-P and MCPA) also appeared at lower levels. Bentazone, DEET and diuron were detected in all samples at low levels. Among the 19 PAHs phenanthrene occurred at the highest concentrations, but fluoranthene, pyrene and naphthalenes also contributed to the total PAH concentration. In the case of the POCIS sampler selective for glyphosate and AMPA, the levels of AMPA metabolite exceeded significantly that of the parent herbicide compound.

Introduction

In the frame of project SIMONA (DTP2-093-2.1) entitled "Sediment-quality Information, Monitoring and Assessment System to support transnational cooperation for joint Danube Basin water management" [1], development and test of a monitoring system was carried out in one of the test areas of the Drava River Basin (at Barcs) in 2020-2021. The project, proceeding with the participation of 17 full partners and 13 associated partners from 14 countries, aims to respond to the current demand for effective and comparable measurements and assessments of sediment quality in surface waters in the Danube river basin by delivering a ready-to-deploy sediment-quality information, monitoring and assessment system to support transnational cooperation for water management in the region. Thus, the main objective of project SIMONA is to achieve an improved, harmonized and coordinated sediment quality monitoring practice in the Danube river basin. For this purpose, a harmonized Sediment Sampling Protocol and a Laboratory Analysis Protocol have been established [2] within the project, and laboratory analysis has also been extended by a passive sampling regime.

The main components of the passive sampling system were (a) sediment box for the systematic collection of suspended particles, (b) sensors for recording different physicochemical parameter (e.g., temperature, turbidity, dissolved oxygen, pH) and (c) passive samplers for uptake of different contaminants. There are numerous commercially available or home-made passive sampler devices (e.g., silicon rubber, SPE disks, POCIS) providing the time weighted average (TWA) concentration of dissolved pollutants, and their sorbent phases are selective for different groups of target components. First we have tested the Polar Organic Chemical Integrative Sampler (POCIS) designed to of hydrophilic organic chemicals (pesticide residues). Next we have applied the POCIS selective for glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) and several SPE disks for sampling of metals, polycyclicaromatic hydrocarbons (PAHs) and pesticide residues.

Experimental

We have used Affinisep passive samplers for the monitoring of different classes of pollutants. The disk-based passive samplers (Chelating, HLB, C18) included in the SIMONA sampling protocol were used to collect metals, pesticides, and PAHs. For glyphosate and AMPA metabolite a selective POCIS phase was applied.

Instrumental analysis was performed at Bálint Analitika Ltd. using liquid chromatography coupled with mass spectroscopy (LC-MS) or gas chromatography chromatography coupled with tandem mass spectroscopy (GC-MS/MS) for determination of 85 target pesticides and a GC-MS (selective ion monitoring, SIM) method for 19 PAH compounds. The results of the measurements refer to the amount of contaminants collected by the entire disk at the current stage of evaluation, and are currently being calibrated to absolute concentrations for the liquid phase. However, based on the scientific literature, data related to the sampling rates (R_s) and time weighted average (TWA) levels of solved pollutants were also calculated in some cases. Suspended sediment samples were collected monthly either in a 30-liter water sample (point sample), or in a standardized sediment box used for long-term sample collection. Contamination

sample), or in a standardized sediment box used for long-term sample collection. Contamination levels were quantitatively determined in both sample types. During the monitoring phase, water temperature, turbidity, dissolved oxygen levels and pH were measured on a continuous basis by electrochemical and photoelectric sensors.

Results and discussion

The point samples collected in the barrel contained little sediment, which significantly limited the reliability of the analytical measurement. In contrast, the sediment box collected and partially separated the suspended sediment by the baffles of the box, allowing the analysis of a significant amount of sample.

We have compared the passive sampling procedures using the binding phase (adsorbent) in the form of a disk and powder (POCIS) during the development of the monitoring procedure in surface water (Drava). According to the results of our preliminary investigations in 2020, the amounts of bound pesticide residues were similar, but in some cases the membrane holding the adsorbent was torn, so the powder was lost, thus we decided to use a disk.

Regarding the pesticide residues, pollution pattern and trends were in accordance of our expectations. Preliminary results prior to the agricultural season indicated that bentazone, DEET and persistent diuron are the main background pollutants. The latter active ingredient is no longer authorized in the EU. The concentrations of terbuthylazine, S-metolachlor and tebuconazole increased significantly during the spring, and then decreased gradually during the summer except of tebuconazole, which was detected only in May. The highest concentrations (1140 ng/sample) were measured for the chloroacetamide type herbicide, S-metolachlor in May, while terbuthylazine from the triazines was present at 439, 83, 19.7 and 14.3 ng/disk sampled in May, June July and August, respectively (see Figure 1). Chlorophenoxy acids appeared later at lower levels. 2,4-D and mecoprop-P concentrations measured in June, were 18.2 and 8.8 ng/sample respectively, whereas only about 1 ng/sample of mecoprop-P and MCPA were detected in July.

Point water samples were collected when the passive samplers were changed in every month. Levels of the pesticide active ingredients measured in these samples were in the range of 1 to 20 ng/L. These values are in the same order of magnitude and similar to TWA concentrations calculated from the sampling rates (R_s) taken from the literature [3,4]. On the basis of these values the calculated highest TWA concentration for terbuthylazine was 23.5 ng/L in May, and the concentration of metolachlor remained under 10 ng/L in the winter.



Figure 1 Amounts of pesticide active ingredients having the highest concentrations

Two of the 19 PAH target compounds, acenaphthylene and dibenzo(a,h)anthracene were below the detection limit in all samples collected. In addition, anthracene was not detected in May, whereas neither benzo(a)pyrene, nor indeno(1,2,3-cd)pyrene were detectable in the sample collected in June. The other compounds were measurable at levels between 0.312 and 35.0 ng/sample. The total PAH concentration measured in the May sample was about twice as high as in June, and the level in July was higher than in May (83 ng/sample). Among the 19 PAHs phenanthrene had the highest concentration, but fluoranthene, pyrene and naphthalenes also significantly contributed to the total PAH concentration (see Figure 2).



Figure 2 Amounts of PAH compounds measured on the C18 disk

In the case of the POCIS sampler selective for glyphosate, the higher concentration of the AMPA metabolite compared to glyphosate was noticeable: 5.5 ng of glyphosate and 126.5 ng of AMPA were collected in March, while 21.8 and 353.5 ng/sample were measured in June.

Conclusion

The use of the disk was proven to be more convenient compared to the POCIS containing sorbent powder between the two membranes. Use of the membrane for the disks decreases the background noise in the chromatogram, but further experiments are required for a more precise assessment of the effect. The test is now in progress regarding the application of the membranes together with the C18 disks as they are present in all samples. We have also observed that the elution procedures provided by the manufacturer are not defined at an expectable accuracy, thus, these processes need to be optimized and fit into the analytical procedure. Therefore, different hydrophilic/lipophilic balanced (HLB) disks were prepared for the LC and GC determinations of pesticide residues.

Similar results were obtained for grab samples (point sample) and from estimations using the sampling rates (*Rs*) from the scientific literature. The current list of priority compounds contains persistent and most toxic components, but for the regular monitoring further compounds should be involved. Among the pesticide active ingredients thiabendazole, azoxystrobin, boscalid, propiconazole, terbuthylazine-desethyl, clomazone, pendimethalin, dimethenamid, pyrimethanil, metrafenone, PBO, thiacloprid and tetraconazole were also detected.

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AN ECONOMIC METHOD OF MICROPLASTIC SEPARATION, EXTRACTION AND IDENTIFICATION IN AGRICULTURAL SOILS Ibrahim Sa'adu ^{1,2}, <u>Andrea Farsang¹</u>

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Abstract

Plastics has become became a major consumable product and alternative in agriculture as a result of its playing role in energy conservation, maintaining of uniform soil temperature, and controls of weeds and fertilizer transport and thereby contaminate the soils. This research aims to provide the cost-effective method for microplastics separation and extraction from the agricultural soils. The soils were randomly collected from the greenhouse farming and conventional agriculture. The plastics used for recovery tests were collected from the field and cut off into pieces. Result from the field shows that density separation with ZnCl₂ using this method has the highest extraction capacity (400 ± 100 pieces/Kg) and recovery rate (90%) compare to other floatation solutions. The method was very effective in extracting both low and high densities microplastics. Furthermore, the results infer that NaCl₂ and distilled H₂O were effective in extracting low densities microplastics such as LDPE and PP. This method provides several alternatives depend on the economy and target of users.

Introduction

Plastic is an indispensable tool in agricultural sector because of its role in processing and handling of agricultural products from nursery, planting to post harvest periods. It became a major consumable product and alternative in agriculture owing to its properties of cheapness, impermeability to precipitation and gases, malleability lightweight, maintaining of uniform soil temperature, and controls of weeds (Sussana, 2018; Patel and Tendel, 2017). The horticultural industries are emerging as major potential consumers of the plastics in form of sheets and films for crop protection, energy conservation, diseases, and pest control, water conservation supply and drainage, fertilizer transport, and building and structures (Patel and Tendel, 2017). Global plastic production has increased from 2 million tons in the 1950s to 359 million tons in 2018, the rate of this plastic recycle is very low (plastic Europe, 2019). More than half is used in protective cultivation such as a greenhouse, small tunnel, mulching, etc. Asia accounts for 48.21%, Europe 18.5%, North America 17.7%, Africa 7.1%, Latin America 4% and 2.6% go to CIS countries. China and Japan witnessed drastic growth in the sector and account for more than 30% of plastic production. Similarly, in India 5 tones of plastics is produced annually and 0.35 million tones go to agriculture (Espejo et al, 2012; Patel and Tendel, 2017).

The sources of plastic contaminants in agriculture come from primary sources such as sewage sludge, organic and inorganic fertilizer application, irrigation water application, atmospheric and wind deposition, etc.(Kaweck, et al, 2021; Wu et al, 2021; Yang et al, 2021; Katsumi et al 2021). Also, the sources can be secondary as a result of larger plastic materials disintegration from mulching, greenhouse films, plastic gauze, etc. (Mo et al 2021, Schothorst et al, 2021; Babagyayou et al, 2020; Huang, 2020). The disintegration is caused by the aging of plastic films as a result of climatic, agrochemical use, and environmental pollution factors(Dehbi, 2015; Alhamdan, 2009). These plastic contaminants litter the municipalities, cities, and farmlands because the rate of degradation is very low. Microplastic waste generated can be transferred horizontally and vertically in the soil by wind, water, microorganisms, and leaching.

The Presence of plastic contaminants causes imbalance to the ecosystem such as soil, plants, water bodies, aquatic lives, underground water, insects, animals, and human health(Serrano-Ruiz et al, 2021; Zhang et al 2021; Rondoni et al, 2021; Li et al 2021; Mora et al, 2021).

However, being the studies of microplastics in the agricultural soil new and emerging(Wang et al, 2021), there is a lack of standard methods on how to identify and quantify the large concentration of microplastics in the soils (Li et al 2019; He et al, 2018). Furthermore, most of the available methods have limitations of use because of their high cost and rigorous nature of preparation stages. Also, some methods (such as Wu et al, 2021; Li et al, 2020; Zhang et al, 2018) consider single polymer type (low-density plastics). This has limitations in the agricultural soils because it comprises different compositions (organic matter, minerals, and clay) and plastic contaminants with different densities. Application of these methods will not be suitable for soils with multiple contaminants of different sizes and densities.

Materials and Methods

Sampling

The validation test was carryout on three different soils from two agricultural farmlands with different land use. The first farmland was subjected to greenhouse farming while the second was subjected to arable farming. The greenhouse farmland was already divided into 15 parcels; each parcel has the same size of 52.30m in length and 9m breadth. Three parcels were randomly selected. At this time each parcel is equally divided into two parts (known as parts A and B). In each part, the soil layer was divided into two layers (0-20cm and 20-40cm). Four samples from the same layers were bulk together and formed one composite sample. The same procedures were followed for the arable farmland. Thus, a total of 20 samples were collected from two different layers of the soils with different land-use type. However, for recovery test, five field plastics contaminants of macroplastics plastics that were use were obtained from the same field. These were cut off to pieces and formed microplastics Laboratory Analysis

This methodology was implemented base on the improvement of the Liu et al (2019) method. The method was developed because of the high cost of other recently developed method among the other reasons. Briefly, the soils were oven-dried at 40° C, sieved with 5mm. A weight of 10g were placed on 250 ml conical flasks, 40 ml of 30% H₂O₂ and 10 mls of Fenton reagent were used for organic matter digestion. The solutions were place of heat sources of 70°C until the solutions were dried up or nearly dry. Immersion of the flask containers to cold water and addition of few drops of butyl alcohol reduced the spout out of the samples. 40 ml of 5mol/L $ZnCl_2$ solution (1.5g/cm³) was used as floatation salt. The solutions were capped with aluminum foil and shaken for 1 hour at 250 rpm in orbital shaker and emptied in 100ml beakers and allowed settling for 24 hours. About 20ml of upper supernatants were pipetted with glass pipette. 20ml of ZnCl₂ were added to the solution and shaken for 30 minutes in the orbital shaker for the second time. This was done in order to effectively remove the microplastics presence in the soils. The upper supernatants were combined with the second one and form a single microplastics extracts. These were later filtered through 20um and 0.45um respectively using vacuum pump. The filters were dried and taken to microscope laboratory for microplastis identification and quantification. The suspected plastic particles were confirmed through; 1. using needle and heat method and 2. Raman spectroscopic analysis.

A.	$\frac{\mathbf{B}}{\mathbf{S/n}}$	Floatation solutions	Sampl (Pieces	e 1 s/Kg)	Sample 2 (Pieces/Kg)	Samı (Piec	ole 3 T es/Kg)	'otal	Mea	n	SD
Soil weight (10g)	1.	ZnCl ₂	300		300	600	1	100	400.	00	173.21
	2	NaI	100		200	500	8	00	266.	67	208.17
Digestion (40ml 30% H ₂ O ₂ +	3	NaCl ₂	100		100	100	3	00	100.	00	00
$10\text{ml FeH}_4\text{O}_6\text{S}^{+2})$	4	H_2O	00		00	200	2	00	66.6	6	115.47
Density Separation Density Separation											
(20ml ZnCl ₂ Repeat)	$\frac{C}{S/n}$	Floatation			MiP(10pi	eces)				Tota	al Recovery
(20ml ZnCl ₃ Repeat) Sedimentation & Pipetting(After 24-h)	$\frac{C}{S/n}$	Floatation solutions	PP	LDPE	MiP(10pi PET	eces)	PVC	PL	J	Tota	al Recovery rate (%)
Comi ZnCl, Repeat) Sedimentation & Pipetting(After 24-h)	$\frac{C}{S/n}$	Floatation solutions	PP Fiber	LDPE Film	MiP(10pi PET Fragment	eces)	PVC Fragmen	PU t Fo	J am	Tota	l Recovery rate (%)
Comi ZnCl ₂ Repeat) Sedimentation & Pipetting(After 24-h) Filtration (20µm& 0.45µm)	<u>C.</u> <u>S/n</u>	Floatation solutions	PP Fiber	LDPE Film	MiP(10pi PET Fragment	eces)	PVC Fragmen	PL t Fo	J pam	Tota	al Recovery rate (%)
Comi ZnCl, Repeat) Sedimentation & Pipetting(After 24-h) Filtration (20µm& 0.45µm) Quantification (Microscope)	<u>C.</u> <u>S/n</u> <u>1.</u>	Floatation solutions	PP Fiber	LDPE Film 10	MiP(10pic PET Fragment	eces)	PVC Fragmen	PU t Fo 5	J pam	Tota	al Recovery rate (%) 90
Coml ZnCl ₂ Repeat) Sedimentation & Pipetting(After 24-h) Filtration (20µm& 0.45µm) Quantification (Microscope)	$ \begin{array}{c} \underline{C.} \\ \underline{S/n} \\ \underline{1.} \\ 2 \end{array} $	Floatation solutions ZnCl ₂ NaI	PP Fiber 10 10	LDPE Film 10 10	MiP(10pic PET Fragment	eces)	PVC Fragmen 10 10	PU t Fo 5 0	J pam	Tota 45 40	Al Recovery rate (%) 90 80
Comi ZnCl, Repeat) Sedimentation & Pipetting(After 24-h) Filtration (20um& 0.45um) Quantification (Microscope) Polymer	$ \begin{array}{c} \underline{C.} \\ \underline{S/n} \\ \underline{1.} \\ 2 \\ 3 \end{array} $	Floatation solutions ZnCl ₂ NaI NaCl ₂	PP Fiber 10 10 10	LDPE Film 10 10 7	MiP(10pic PET Fragment	eces)	PVC Fragmen 10 10 0	PU t Fo 5 0 0	J pam	Tota 45 40 21	d Recovery rate (%) 90 80 42
Comi ZnCl, Repeat) Sedimentation & Pipetting(After 24-h) Filtration (20um& 0.45um) Quantification (Microscope) Polymer Identification (Raman/ FTIR)	$ \begin{array}{c} \underline{C.} \\ \underline{S/n} \\ \underline{1.} \\ 2 \\ 3 \\ 4 \end{array} $	Floatation solutions ZnCl ₂ NaI NaCl ₂ H ₂ O	PP Fiber 10 10 10 10	LDPE Film 10 10 7 9	MiP(10pic PET Fragment 10 10 4 0	eces)	PVC Fragmen 10 10 0 0	PU t Fo 5 0 0 0	J bam	Tota 45 40 21 19	al Recovery rate (%) 90 80 42 38

Figure 1.Extraction method and results. (A); Schematic diagram of the method. (B); Validation of the method of 4 floatation solutions. (C); Recovery test using different floatation solution on different microplastics densities

Result and Discussion

Microplastics were detected in all soils tested with different floatation solutions. Table a. Shows that; $ZnCl_2$ and NaI yielded higher MiP concentration of 400 ±100 pieces/Kg and 266.67± 120 pieces/Kg respectively. Also, NaCl₂ and distilled H₂O recorded the low average concentration of 100 pieces/Kg and 66.66 pieces/Kg respectively. Similar findings were reported in the method developed by Li et al, (2019) where $ZnCl_2$ and NaI reported to have the excellent yield of microplastics extraction compare to other salts. However, the recovery test by Table b. shows that $ZnCl_2$ has the highest recovery rate of 90% followed by NaI which has 80%. These recoveries conform to findings of Wu et al, (2021) and Li et al, (2019). Furthermore, the careful observation of the table shows that all the floatation solutions tested good for low density plastics (PP and PE) as all the low densities were recovered in high number. But for the high density plastics (PET, PVC and PU), high recovery rates were only found in the samples treated with ZnCl₂ and NaI solutions. This result confirmed the findings of Zhang et al, (2018) which concludes that density separation with NaCl₂ was efficient in extracting low density plastics such as PP and LDPE.

However, the recovery tests reveal capacity of floatation solutions on plastic structure. ZnCl₂ and NaI were tested very well in extracting fibers, film, and fragment. But the ZnCl₂ yielded average result (5 pieces) in terms of foam's extractions while the NaI was recorded very low in terms of foam structures. The reason of low recovery of PU (foam) despite its less density compare to PET and PVC might be associated to the nature of foam materials of larger pore space that were occupied by soil particle materials and increases it density. Similarly, for NaCl₂ and distilled H₂O, only fibers and films were recovered at the high rate. This finding also tally

with several findings which concluded that these salts solutions are efficient in removal of fibrous materials (Liu et al 2018; Corradini et al, 2019; Li et al, 2019)

Conclusion

This method tests the extraction capacity of different floatation solutions on low and high density micropplastics. The method was developed to minimize the cost of microplastic extraction analysis. In both the validations and recovery tests, the method shows very good result with ZnCl₂ and NaI for the separation and extraction of high and low density plastics particles as well as all the plastic structure with the exception of foam. Similarly, for the extraction of low density microplastics as well as structures such as fiber and film, NaCl₂ and distilled H₂O can serve as good floatation solutions.

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THE EFFECT IF EXTRACTION TIME AND SAMPLE RATIO ON THE PROCESS OF EXTRACTING ACTIVE SUBSTANCES FROM HAWTHORN (CRATAEGUS MONOGYNA) USING ULTRASOUND

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Abstract

Ultrasound-assisted extraction (UAE) is one of the modern method that is applied today with the aim to decrease solvent consumption, shorten the extraction time, increase the extraction yield, and enhance the quality of extracts. In this paper, the effect of extraction time and the ratio of the sample to the solvent have been studied on the ultrasonic extraction of hawthorn fruits. The hawthorn fruit was collected from Normafa, Hungary. 10% ethanol and pure distilled water were used as extraction solvent. Folin-Ciocalteu method was performed to visualization of total phenolic compounds (TPC) and the ferric reduction antioxidant power (FRAP) method to quantify antioxidant activity in the extracts. Analyzing the results by two-way-ANOVA at significant level p < 0.05 by using SPSS software demonstrated that there is significant effect of time of extraction and the ratio of the sample to solvent.

For TPC the best value was obtained at (9 min, 0.05 g/ml sample-solvent ratio) with a mean $(451.106 \pm 0.79 \text{ mg/L})$ by using 10% ethanol, and at (3 min, 0.075 g/ml sample-solvent ratio) with a mean $(365.289 \pm 15.96 \text{ mg/L})$ by using distilled water. While the best antioxidant activity was with a mean $(223.033 \pm 1.01 \text{ mg/L})$ at (9 min, 0.075 g/ml sample-solvent ratio) and $(50.678 \pm 2.02 \text{ mg/L})$ at (6 min, 0.1 g/ml sample-solvent ratio) by using the 10% ethanol and distilled water respectively. More work is needed to define better conditions for using the distilled water as a solvent in the ultrasonic extraction.

Introduction

Hawthorn is a common name of all plant species in the genus Crataegus, that is belonging to the Rosaceae family, the size of the trees ranged between shrubs and trees that can reach a height of up to 10 m. Normally has bright green leaves, white flowers, and bright red berries, each containing one to three or five seeds, depending on the species. Most of the species grow in North America as well as in East Asia, Europe especially Turkey [1,2]. The fruits of hawthorns are also edible, as most people who have tried them will testify. The colour of the ripe fruit ranges from yellow, through green to red, and on to dark purple. Most of the species ripen their fruit in early to mid-autumn [3]. The leaves, flowers, and berries of hawthorn contain a variety of flavonoids which include oligomeric procyanidins, vitexin, quercetin, and hyperoside. Other chemical constituents include tannins, nitrogen-containing compounds, triterpenoids such as ursolic acid, $2\alpha, 3\beta, 19\alpha$ -trihydroxyursolic acid, corosolic acid, cycloartenol, uvaol, oleanolic acid, crataegolic acid, butyrospermol, 24-methylene-24dihydrolanosterol. Table 1 is showed the chemical constituents of hawthorn fruits [4].

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Chemical constituents	The amount	Reference
Flavonoids	0.1-1%	
Oligomeric proanthocyanidins	1-3%	Г1 1
Triterpene acids	0.5-1.4%	[1]
Organic acids	2-6%	
Epicatechin	178.3 mg/ 100g dry fruit	
Chlorogenic acid	69.4 mg/ 100g dry fruit	
Hyperoside	24.6 mg/ 100g dry fruit	
Isoquercitrin	13.4 mg/ 100g dry fruit	[5]
Protocatechuic acid	3.2 mg/ 100g dry fruit	
Rutin	2.6 mg/ 100g dry fruit	
Quercetin	0.9 mg/ 100g dry fruit	

Table 1: chemical constituents of hawthorn fruits.

In folk medicine, hawthorn fruits have been used as a cure against stress, nervousness, sleep disorders, heartache, stomachache, and sore throat [6]. Nowadays hawthorn extracts are among the most popular herbal medicinal products in many European countries and the USA after getting attention due to its potential cardiovascular enhancing and protective properties, where In Germany, hawthorn extract has been approved and registered as a therapeutic agent for the treatment of minor forms of coronary heart disease and congestive heart failure. The current study suggested that hawthorn could be used as an alternative therapy for various cardiovascular diseases, such as angina, hypertension, hyperlipidemia [7].

Materials and methods

- 1) Hawthorn fruit was collected from Normafa, Hungary.
- 2) Ethanol (≥99 %, REANAL LABOR, Co., Hungary) was used for extraction purpose.
- 3) The applied chemicals and reagents for the analysis were collected per following list: methanol (LACH-NER, Co., Czech Republic), gallic acid (SIGMA-ALDRICH, Co., USA, product of China), acetic acid (96 %, REANAL LABOR, Co., Hungary), Lascorbic acid (reagent grade, SIGMA-ALDRICH, Co., USA, product of China), hydrochloric acid (37 %, CARLO ERBA Reagents S.A.S, France), Folin-Ciocalteu reagent (SIGMA-ALDRICH, Co., USA), sodium acetate (Analar grade, VWR Chemicals, Belgium). 2,4,6-Tris(2-pyridyl)-s-triazine (≥99 %, SIGMA-ALDRICH, Co., USA), sodium carbonate (MERCK KGaA, Germany), ferric chloride (REANAL LABOR, Co., Hungary).

Raw materials and extraction methods:

After removing the sticks and the crushed fruit, Hawthorn fruit was washed and cleaned, and dried from the water, then Shredding of the different portions was done using GM 200 pulverizer, the (UAE) process was performed by using (ULC PREMIUM,WEBER ULTRASONICS AG, Germany) with power 100 W this apparatus available in livestock and food preservation technology department, during (3, 6, 9 minutes), and by using (0.05. 0.075, 0.1 g/ml sample-solvent ratio) by using 10% ethanol and distilled water.

Total phenolic compounds (Folin-Ciocalteu method):

Total phenolic compounds (TPC) were estimated by the Folin-Ciocalteu method [8]. The absorbance was measured at 760 nm Gallic acid (0.3 mM) was used as the standard and the amount of TPC was calculated as: TPC = $(A \cdot 2500 \cdot DF)/(S \cdot a) [(mg GAE)/L]$ Antioxidant activity (AA) assays (FRAP Method):

The ferric reduction antioxidant power (FRAP) method was applied to quantify antioxidant activity. Reading the absorbance at 593 nm was performed after exactly 5 minutes of incubation. The calibration was realized with ascorbic acid (10 mM). The calculation was done using the following equation: $AA = (A \cdot 1550 \cdot DF)/(S \cdot a) [(mg ASE)/L]$

Where A is the absorbance; S is the amount of sample (μ L); a is the slope of the calibration curve; DF is the dilution factor.

Statistical analysis:

All experiments were repeated twice. Two-way analysis of variance and Tukey's test with 95% confidence level were employed to compare the significant differences among results using software SPSS (version 27.0).

Results and discussion

Total phenolic compounds:

As shown in Figure 1, samples extracted by using 10 % ethanol at (9 min, 0.05 g/ml samplesolvent ratio) gave the highest total phenolic content (TPC) with the mean yield (451.106 \pm 0.79 mg/L), and lowest extraction efficiency was at (3 min, 0.05 g/ml sample-solvent ratio) with the mean yield (80.453 \pm 1,99 mg/L) while the other samples converged, and their average ranged between (132.678 - 273.261 mg/L).

Total phenolic content (TPC) was lower when the distilled water was used as shown in figure 2, the highest yield was obtained at ((3 min, 0.075 g/ml sample-solvent ratio)with the mean $(365.289 \pm 15.96 \text{ mg/L})$.



Fig 1: Effects of extraction time and sample ratio on the yield of TPC using 10% ethanol.

Fig 2: Effects of extraction time and sample ratio on the yield of TPC using distilled water.

Antioxidant activities (AA) assays:

The results of the FRAP analysis (figure 3) showed that the highest antioxidant activity by using 10% ethanol was at (9 min, 0.075 g/ml sample-solvent ratio), with mean (223.033 \pm 1.01 mg/L) while the lowest activity was at (3 min, 0.05 g/ml sample-solvent ratio) with mean (66.9334 \pm 0.67 mg/L). It can be seen that the activity of the antioxidants increased with the increase in the extraction time, regardless of the percentage of the sample.



Fig 3: Effects of extraction time and sample ratio antioxidant activity using 10% ethanol.



Fig 4: Effects of extraction time and sample ratio antioxidant activity using distilled water.

The figure 4 showed that the highest antioxidant activity by distilled water was at (6 min, 0.1 g/ml sample-solvent ratio), with mean ($50.678 \pm 2.02 \text{ mg/L}$) as well as at (3 min, 0.075 g/ml sample-solvent ratio) the antioxidant activity was very close with mean ($50.2 \pm 1.35 \text{ mg/L}$), while the lowest activity was at (6 min, 0.05 g/ml sample-solvent ratio) with mean ($13.864 \pm 0.67 \text{ mg/L}$).

It can be seen that extraction time has a clear effect on the total content of phenols and the activity of antioxidants. It is expected that it increases with the increase in the extraction time, but the extraction time is a critical parameter in the extraction process, and it has a complex effect. Where during the extraction process, the solvent needs time to diffuse into the cell, and then the analytes react with the solute and dissolve into the extraction solvent, finally, the target compounds diffuse out of the matrix and transfer into the liquid phase. Therefore, the extraction time depends on the time required for the dissolution and diffusion process. The possible reason might be that inadequate extraction time would cause incomplete neutralization for the analytes and insufficient dissolution and diffusion. However, too long an extraction time would lead to structural destruction and decomposition of target analytes [9]. Also, the effect of the sample ratio and its surface area can be explained by this multi-stage process for the diffusion of the solvent and its ability to extract.

Different techniques and solvents, such as ultrasound, microwave, supercritical fluid, leaching, and heating under reflux, have been introduced to extract bioactive substances from the Hawthorn plant. [10] concluded that deionized water extract and 80% acetone extract showed better antioxidant activities compared to ethanol 80% and methanol 80% and that deionized water extract showed a significantly different FRAP from other three extracts during using the UAE, while [11] indicated milk and whey were better than distilled water in (Tannins, Pectic substances, Food fibers) extraction process at different temperatures.

Conclusion

The effect of three times (3, 6, 9 minutes) and three different percentages for the sample (0.05, 0.075, 0.1 g/ml sample-solvent ratio) were studied, and the results indicated a significant effect of each on the extraction process. By measuring the total content of phenols, it was found that the best content (451.106 \pm 0.79 mg/L) and (365.289 \pm 15.96 mg/L) were obtained at (9 min, 0.05 g/ml sample-solvent ratio), (3 min, 0.075 g/ml sample-solvent ratio) using 10% ethanol and distilled water, respectively. The extracts gave the highest oxidation activity at (9 min, 0.075 g/ml sample-solvent ratio) with a mean (223.033 \pm 1.01 mg/L) by using 10% ethanol, while the best value was (50.678 \pm 2.02 mg/L) at (6 min, 0.1 g/ml sample-solvent ratio) using distilled water. according to the results, it can be said that it is possible to use distilled water as a solvent to extract the active substances, but it needs further study and the use of other conditions to reach better conditions for the extraction process.

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MECHANOCHEMICAL ACTIVATION OF KAOLINITE TO MANIPULATE THE DISSOLUTION KINETICS OF THE SOLID

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Abstract

The well-established Bayer process is the primary means of alumina production from bauxite; however, it generates more than 2.7 billion tons of bauxite residue (commonly called red mud) annually [1]. This quantity can be reduced by applying the so-called desilication step before the extraction of alumina at high temperature. This is a commonly used method when the bauxite ore contains high concentrations of silica. Bauxites contain silica in various forms, some of them are rather reactive and are capable of dissolving under typical Bayer digestion conditions, causing major caustic and alumina losses, scale formation in the pipes and exerting unfavorable effect on the quality of the final product. Our main goal was to contribute to the control of these processes by investigating the chemical events that take place during desilication.

Kaolinite is the main reactive silica containing clay mineral in bauxite. During desilication, it transforms into mixed sodalite (that is, different anions present in the product), called DSP (desilication product) [2]. In order to gain more information about the dissolution of the kaolinites and the subsequent formation of DSP in these systems, a suitable apparatus was designed, and the processes were investigated under conditions largely mimicking the industrial ones (using sodium aluminate solution with specific concentrations of the spent liquor and carrying out experiments at 95°C). To identify the key parameters responsible for the kinetics of the dissolution, the raw kaolinite was ground to change the characteristics of the starting material, hence affecting the dissolution rate [3]. The concentration of the Si(IV) during the dissolution-DSP formation process was monitored with ICP-OES, and the starting and final materials were characterized by a variety of experimental techniques.

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POTENTIAL SOURCES, SYNTHESIS METHODS AND INDUSTRIAL APPLICATIONS OF WASTE GENERATED GRRAPHENE-BASED NANOCOMPOSITES

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One of the most urgent worldwide problems is waste treatment, burdening all human activities from households to large industries, which increases in parallel with the overpopulation. In this presentation, we overview different type of waste sources which can be easily converted to carbon particles for large scale composite production by nanotechnology. In the past decade, graphene-based nanocomposites and carbonaceous composite materials gain significant interest in terms of potential environmental applications. Due to its versatile composite and film forming capabilities, graphene-based materials such as graphitic carbon, graphene, grapheneoxide and reduced graphene-oxide can act both as matrix or filler in composite materials. Moreover, the sp^2 hybrid state of carbon atoms in the honeycomb like lattice structure prove remarkable electric and conductive characteristics, which can be heavily influenced by the degree of oxidation matrix-filler ratio and the type of the other components. In addition, carbonaceous materials are applied to improve the mechanical properties of structural materials for long time. Traditional applications such as membrane filtering or adsorption processes can be also built on waste-derived graphene based nanocomposites. In terms of electrical applications, due to the bandgap influenced by the composition the photocatalysis is also a potential way to reuse graphitic materials. Besides this interesting approach, we highlight how the valorization of waste materials can prove a cost-effective and benign solution in the green synthesis of various carbon based nanocomposites.



Fig.1. Illustration of the main classes of graphene energetic nanocomposites.

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INVESTIGATION OF ENANTIOSELECTIVE HPLC SEPARATIONS OF FLUORINATED β^3 -PHENYLALANINE DERIVATIVES

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Many chiral compounds are involved in processes that take place in living organisms. Typically only one enantiomer of the image-mirror image pairs of chiral molecules occurs in nature, such as the L-enantiomers in case of amino acids. The two members of the enantiomer pairs may have significantly different biological effects, so it is important to either synthesize the pure enantiomers or separate the racemate mixtures.

The L-enantiomer of phenylalanine, a biologically active amino acid, can better (than the Denantiomer) cross the blood-brain barrier and is involved in the synthesis of neurotransmitters with antidepressant and analgesic effects in human body. It also plays a significant role in biosynthesis of flavonoids in plants.

Studying the bioactivity of drugs after the replacement of certain atoms or functional groups with fluorine became an intensively researched topic in the second half of the last century. Fluorine-containing active ingredients were only present in 2% of pharmaceutical medicines in the '70s, while it can be estimated at around 25% nowadays. Incorporation of fluorine can alter many of the properties of the compounds, such as the steric interaction of the functional groups, lipophilicity or the ability to make H-bridge interactions, thus increasing the efficacy of a drug by orders of magnitude.

In this study, ion-exchanger type chiral stationary phases were utilized to separate fluorinated β^3 -phenylalanine derivatives. We examined the effect of mobile phase composition, quality of the base additive, concentration of acid and base additives, and temperature on the chromatographic properties. The elution order was determined in all cases. We also studied the relationships between structural peculiarities, retention and thermodynamic parameters.

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ETHICAL FOUNDATIONS BEHIND SOCIAL RESPONSIBILITY: A STUDENT SURVEY

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Abstract

The path pf preventing and solving the environmental problems in the world is surrounded by innovations, new technologies. However, it should not be overlooked that these are aimed at human well-being and are mainly coordinated by companies. People make decisions for people but along with different interests. Understanding the personal values behind the business decision can move forward a more responsible approach to business. The paper presents 100 government and 100 business student's opinions about business ethics by selected questions of the ATBEQ questionnaire. The results show that self-sacrifice is not kept immoral by the students, and morality can go beyond self-interest. However, the primary responsibility of companies is making money. There are few significant differences found between government and business students, but the distributions of the responses suggest different patterns of thinking.

Introduction

The main source of environmental problems is social [1]. We are meeting an ever-increasing level of needs led to new technologies in agriculture, mechanical engineering, health care, or even education. New materials and new production technologies often upset the balance of nature and lead to environmental pollution. Technological and management efforts are more sophisticated but apparently not enough. Assuming that novel technologies aim for the higher satisfaction of consumer needs and considering that both purchasing and manufacturing decisions are made by people, solutions also must be found in minds. Environmental consciousness can be improved on an individual level [2], and there has been a particular emphasis on social responsibility at the corporate level for decades. Several models and approaches have been developed in the field to understand the motivations and limitations of environmentally and socially friendly behaviors. Carroll's pyramid [3] distinguishes four levels of responsibility, including economic, legal, ethical, and philanthropic ones. The review of the model in 2016 [4] confirmed that ethics permeates these levels. Economic and legal levels are marked 'required by the society'. Henceforth, ethical responsibilities are marked as expected and philanthropic level as desired. An ethical approach is presented in the early definition of Bowen about the social responsibility of a businessman: it "refers to the obligations of businessmen to pursue those policies, to make those decisions, or to follow those lines of action which are desirable in terms of the objectives and values of our society" [5].

According to environmental protection, social responsibility, or ethics, a focus on business students is mainly justified by the fact that they will be the company decision-makers in a few years. Understanding their preparedness allows for an improvement in higher education. The Attitudes Towards Business Ethics Questionnaire (ATBEQ) is an instrument oriented towards attitudes on selecting business ethics situations [6] based on the work of Stevens [7]. The curiosity of ATBEQ is that it is specially applied for business students worldwide. It is a

commonly accepted research instrument in social and management science [8]. Since the change of values is a slow and complex process, the method is applicable for exploring the fundamentals of the approach of business students to business ethics.

Clark et al. [9] give a comprehensive summary of the five moral philosophies which used to provide the basis for empirical investigations:

- "Machiavellianism: Business firms are a self-contained organism with its own 'natural laws' which can be bent but not broken.
- Ethical relativism: Deals with feelings and attitudes and is based on social convention, which accepts behavior sanctioned by established group norms. Hence, moral good means conforming to the way things are in a given time and place and selfishness becomes the root of all evils.
- Legalism: Doing an action or series of actions defensively out of a sense that doing it this way will promote the impression that a practitioner is 'thorough' and 'covered' from an imagined legal point of view.
- Social Darwinism: Individuals, by pursuing their own selfish interests, unwittingly and effectively promote the social welfare. Businesses survive because they obey the natural laws of competition.
- Moral objectivism: The moral guide is rational self-interest which includes the right to defy fate by making rational decisions which lead to productivity and happiness." [9] (p.127)

Experimental

The result of the paper is based on a voluntary online survey among Hungarian Higher education students. The survey questionnaire includes the ATBEQ questions based on [10]. The analysis highlights seven statements (noted the moral philosophy it belongs to). Since Machiavellianism is the most characteristic among Hungarian students in the database, questions about it and self-interest are in focus:

- The only moral of business is making money (Machiavellianism).
- Moral values are irrelevant to the business world (Machiavellianism).
- "Business ethics" is a concept for public relations only (Machiavellianism).
- A business person cannot afford to get hung up on ideals (Machiavellianism).
- True morality is first and foremost self-interested (Moral objectivism).
- Self-sacrifice is immoral (Moral objectivism).
- You should not consume more than you produce (Moral objectivism).

The respondents were asked to mark their agreement on a 5-point scale between totally disagree (1) and totally agree (5). The results are represented with the mean values of the evaluations in the figures. Analysis of variance uses the non-parametric Kruskal-Wallis H test. The significance level is observed at 5%. The data collection period ran from fall 2018 to summer 2020. The survey was managed by Evasys Survey Automation System; data analysis is supported with IBM SPSS version 25. 100 business and 100 state science students are randomly selected from the database for the analysis. Both sub-samples consist of 50 male and 50 female respondents. The representativeness of the sample is not checked.

Results and discussion

According to most of the selected questions, the opinions of government and business students are close to each other (Figure 1). Students of government studies agree with the statement that the only moral of business is making money and consuming must be limited at the incomes more than business students. Significant differences (Table 1) were found about the self-

interested nature of morality; business students accept it more than government students. Self-sacrifice is generally accepted as a moral act, especially among government students.





Although the mean values do not differ remarkably between the student groups, the distribution of the responses on the 5-point scale shows the differences of opinions (Figure 2). According to the money making-nature of the business, the agreement level is stronger among the government students. True morality is first and foremost self-interested is rather agreed (score 4 or 5 marked) by 15% of government students and 37% of business students. The immorality of self-sacrifice is totally refused (score 1 marked) by 47% of the former and 31% of the latter students.



A) Students of government studies

Figure 2. Distribution of results by student groups

Literature in the field often points out that females are more sensitive to environmental problems [11]. Figure 3 shows the results by study type and gender; significant results are marked in Table 1. Male respondents marked higher scores for issues that describe the self-interest of business. Furthermore, female students' scores are higher about the statements that we should not consume more than we produce, especially among business students.



Figure 3. Results by gender

	Government and business		Geno total sa	Gender, total sample		Gender, government		Gender, business	
	stuc	lies			studies		studies		
	K-W	Sig.	K-W	Sig.	K-W	Sig.	K-W	Sig.	
	Η		Н		Н		Н		
The only moral of business is	.42	.517	5.472	.019	7.891	.005	.396	.529	
making money									
Moral values are irrelevant to the	.343	.558	4.604	.032	.989	.320	4.159	.041	
business world									
"Business ethics" is a concept for	.082	.774	6.775	.009	9.741	.002	.405	.524	
public relations only									
A business person cannot afford to	.835	.361	8.137	.004	2.337	.126	6.78	.009	
get hung up on ideals									
True morality is first and foremost	5.9	.015	.008	.930	.24	.624	.064	.800	
self-interested									
Self-sacrifice is immoral	6.39	.011	3.175	.075	4.616	.032	.364	.546	
You should not consume more than	.402	.526	1.342	.247	.028	.867	2.224	.136	
you produce									

Table 1. Results of non-parametric analysis of variance (significant results in bold)

Conclusion

Considering that personal values and culture affect behavior, improving environmentallyconscious actions requires the understanding of the mindset. It is a complex challenge; the analysis of moral philosophies provides an essential point of reference for this. The analysis presented in this paper allows some warning signs for shaping the mind of the future decisionmakers:

• It is encouraging that self-sacrifice is not felt immoral by the students, and morality can go beyond self-interest. Nevertheless, most of them cannot let go of the idea that the business is only for making money.

- The results confirm that females are more sensitive to social issues than males; the latter group presents a more business-oriented approach.
- The difference between government (as future policymakers) and business (as future company decision-makers) students are not significant in most questions of the analysis, but the distributions of the responses suggest different patterns of thinking. Business students are slightly more critical to the social responsibility of companies.

Accordingly, targeted interventions seem to be necessary to achieve better convergence of views of the groups and to strengthen the purpose of the concept of corporate social responsibility.

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A SIMPLE METHOD TO BUILD CATALYST LAYERS FOR THE SYNTHESIS OF VERTICALLY ALIGNED CARBON NANOTUBES

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Abstract

Nowadays, environmental protection and sustainability are getting more and more attention. Thus, our aim was to develop a cost and energy efficient catalyst layer building method for the synthesis of carbon nanotube forests. A simple spray coating method was used to develop a catalyst layer on the surface of the titanium substrates. Then vertically aligned carbon nanotubes (VACNTs) were synthesized directly on the substrate *via* catalytic chemical vapor deposition (CCVD) method. During our research, the effect of catalyst layer deposition parameters on the structure of CNTs was investigated and characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Raman spectroscopy.

Introduction

Vertically aligned carbon nanotubes (VACNTs) have been in the focus of intense research over the years due to their remarkable mechanical and chemical properties such as exceptional electrical and thermal conductivities. VACNTs and their composites are also getting more attention in environmental engineering applications based on their features, for example, sorption capacity, component separation and catalytic activity as well [1,2]. Catalytic chemical vapor deposition (CCVD) is a favored method for the mass production of CNTs it is cost-efficient and simple to use, moreover, it is the only suitable process to synthesize VACNTs [3]. Comparing the possibilities for the production of VACNTs, there are several methods for the formation of thin catalyst layers (i.e., atomic layer deposition (ALD), physical vapor deposition (PVD), pulsed laser deposition (PLD), magnetron sputtering (MS), dip-coating etc. [4,5]) which are able to control layer thickness and morphology, however, they require rather expensive instruments. Among these methods, dip-coating might be an exception, because it requires less complicated instruments, thus, it is widely used for catalyst layer deposition. Spray coating might be an even cheaper method than dip-coating to build thin layers and it also has more variations (thermal, plasma, manual etc. [6,7]). In this research our aim was to study the efficiency of manual spray coating for building bimetallic catalyst layers containing iron and cobalt. Then, their applicability for VACNTs synthesis via CCVD was investigated, which was carried out on the surface of titanium substrates. To gain knowledge about the efficiency of manual spray coating in this specific application, the parameters of the layer formation (substrate pretreatment, coating temperature and number of spraying cycles) were investigated.

Experimental

During the synthesis, a titanium substrate was used to build up the catalyst layers with a spray gun, also used for car painting or art drawing. The concentration of catalyst ink was 0.11 M and the ratio of Fe:Co was 2:3. The latter was prepared from Fe(NO₃)₃×9H₂O and Co(NO₃)₂×6H₂O precursors that were dissolved in absolute ethanol. The Ti substrate was heat treated for 1 h at 400 °C before and after the deposition of the catalyst. This was carried out in a static oven to form a native TiO₂ layer on the surface and oxidize the catalytic particles. The TiO₂ layer might block the diffusion of catalyst particles into the substrate. In some cases, heat treatment process was changed. To prepare thin layers, the pretreated Ti substrate was placed on a heated plate (120-200 °C), then the catalyst ink was sprayed onto the substrate surface. For this purpose, compressed air and a spray gun was used that was operated at constant speed and distance. In one spraying cycle the ink was sputtered 10 times from both directions. The whole process was repeated 5 times (5×10) applying a 30 second-pause to evaporate the solvent from the surface. For the growth of VACNTs, CCVD was used during which the gas feed contained ethylene (70 cm³/min) as carbon source, nitrogen (50 cm³/min) as carrier gas, hydrogen (50 cm³/min) for reductive environment and water vapor (30 cm³/min) for prolonging the activity of catalyst particles. The synthesis time was 35 min and the temperature was 700 °C. The scheme of the production of VACNTs is presented in Fig. 1.



Figure 1. The scheme of manual spray coating layer deposition and production of VACNTs via CCVD

Results and discussion

Since the parameters of the catalyst layer significantly affect the growth VACNTs during CCVD synthesis, they were examined during this work. The effects of heat treatment of Ti substrate, spraying temperature on the properties of VACNTs growth, and number of sputtering cycles were investigated in detail.

In the first sample series, the effect of heat treatment of Ti substrate was investigated during the fabrication of catalyst layer. For this purpose, four different samples were prepared: heat treatment a) before and after – as a reference –, b) only before, c) only after spray coating, and d) without any treatment (Fig.2). VACNTs were grown successfully in the first three cases, while in the fourth case only amorphous carbon was deposited onto the surface of Ti substrate. Heat treatment before spray coating forms a native titania layer that might allow the better adhesion of catalyst ink to the surface. At the same time, heat treatment after spray coating stabilizes the catalyst layer by converting metal nitrate layer into metal oxid layer. The Raman spectroscopy results indicated that heat treating the substrate was beneficial for the graphitic

properties of VACNTs. For the verification of carbon deposit, samples were investigated by SEM (Fig. 2).

The thickness of the catalyst layer can be controlled by the number of spraying cycles, which also influences the formation of separated catalyst particles. Therefore, the number of spraying cycles were varied between 1, 2, 3, 4, 5 and 10 cycles. SEM measurements showed that 1×10 spraying could not provide enough catalyst particles on the surface for the growth of VACNTs. However, applying 10×10 spraying cycles yielded too many catalyst particles that merged together resulting in thick carbon fibers grown on the surface. Applying 2×10 or 5×10 spraying cycles resulted in VACNTs with 8.1 µm or 12.2 µm of height, respectively.



Figure 2. SEM images of VACNTs grown on different heat treated Ti substrates and their corresponding heights

The TEM images also verified that VACNTs grown on the surface of Fe:Co = 2:3 and 5×10 spraying cycles catalyst layer show good graphitic properties with only few defect sites in their walls, which is in good agreement with Raman spectroscopy results.

Finally, the effect of temperature during spray coating on the formation of VACNTs was also investigated. During spraying, the Ti plate was heated between 120-200 °C to evaporate absolute ethanol rapidly from the surface, on which the catalyst particles were uniformly deposited. VACNTs only formed at 120 °C and 140 °C; the corresponding SEM images and height distributions of these samples are shown in Fig. 3.



Figure 3. Height distribution and SEM images of VACNTs synthesized at different spraying temperatures

The evaporation rate above 140 $^{\circ}$ C was too high, thus no homogenous catalyst layer was formed on the Ti substrate.

Conclusions

In summary, spray coating process was proved to be a suitable method for the formation of thin catalyst layers during the production of VACNTs. Heat treatment of Ti substrate, especially after catalyst layer deposition, is needed to sustain stable catalyst layer and synthesize VACNTs with satisfactory orientation. The optimal number of spraying cycles to obtain VACNTs on the surface of Ti substrate was 5 cycles. Increasing the temperature to 140 °C during spray coating can result in higher VACNTs with better quality.

Acknowledgements

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INFLUENCE OF INORGANIC CONSISTUENTS ON PHOTOCATALYTIC DEGRADATION OF IBUPROFEN

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Abstract

Non-steroidal anti-inflammatory pollutants such as ibuprofen are continuously introduced in water media through various environmental routes. Due to their variability in physico-chemical properties, characteristics of sludge used in secondary treatment and other features, pharmaceutical residues are partially removed in conventional wastewater treatment plants. The effect of inorganic constituent (nitrates) present in real aquatic matrices was examined to assess the overall efficacy of the photocatalytic removal of ibuprofen by nanostructured mixture ZnO/SnO₂.

Introduction

Pharmaceutical active compounds (PhACs) represent one of most dominant group of micropollutants which are continuously introduced into aquatic media, primarily through untreated or inadequately treated wastewater. After usage, the pharmaceuticals undergo to different reactions in which they are transformed into metabolites. These metabolites are often more polar and persistent than the parent compounds. Numerous studies have confirmed that ibuprofen exhibits negative ecotoxicological effects on various aquatic species [1,2]. The aim of this study was to investigate the impact of nitrate ions on photocatalytic degradation of ibuprofen.

Experimental

The synthesis of nanostructured materials was carried out using mechanochemical solid-state method. Initial precursors (ZnO, and SnO₂, purity 99.9%) were grounded in an agate mortar for 10 minutes in a molar ratio of 2:1, then annealed in furnace for two hours at 700 °C and once more grounded for 10 minutes [3,4].

Photocatalytic experiment was performed on laboratory scale. A mercury high pressure lamp of 125 W was used as the radiation source, (manufacturer Philips, HPL-N) emission spectrum in the UV range at 304, 314, 335 and 366 nm with maximum emission at 366 nm. The initial concentration of nanostructured mixture ZnO/SnO₂ and analyzed pharmaceutical (ibuprofen) was 0,40 mg mL⁻¹ and 5 mg L⁻¹, respectively. In order to demonstrate the effect of inorganic ions on overall efficiency of ibuprofen decomposition, the nitrate ions were selected. At certain time intervals, 1 mL of treated sample was filtrated through 0,45 µm syringe filters in order to remove nanoparticles. Detection in changes in the ibuprofen concentration is followed by the application of high-performance liquid chromatography (HPLC, Agilent 1260). The concentration of nitrate used for experiment was in the range of 5 to 20 mg L⁻¹. 10 mL of samples were filtered and quantitatively transferred to UV-VIS cuvettes. HACH NitraVer 5 reagent was added to the cuvette containing the sample. The concentration of nitrate ions was determined on UV-VIS spectrophotometer (DR5000, HACH, Germany).

Results and discussion

The obtained results are shown in the Table 1. In order to compare the effect of nitrate ions on the decomposition of ibuprofen, a pseudo first-order constant was used.

Initial concentration of nitrate ions (NO3 ⁻ , mg L ⁻¹)	Rate constant (k, min ⁻¹)
0	0,045 (3)
5	0,0140(4)
10	0,0070(6)
20	0,0070(5)

	- ~					
Table 1	Influence of	f nitrata ione	on photo	potalytic dag	radation of	ibunrofon
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				2 1		

By increasing the concentration of nitrate ions, a significant decrease in the value of the degradation constant was recorded. The decrease in the value of the degradation constant was 85 % when analyzing concentrations of 10 and 20 mg L^{-1} . The inhibitory effect during the photocatalytic process is caused by an increase of NO_3^- charge, which emphasizes the electrostatic repulsion between ions and leads to reducing the rate of active adsorption sites on the surface of photocatalysts [5].

Conclusion

Pharmaceutical compounds are micropollutants which are continuously introduced in water streams considering their inefficient removal by conventional wastewater treatment. The heterogenous photocatalytic treatment by newly synthesized nanomaterial ZnO/SnO_2 for removal of nonsteroidal anti-inflammatory pollutants is demonstrated to be efficient. According to obtained results, the inhibitory effect on ibuprofen removal was proven by increasing the concentration of nitrate ions.

Acknowledgements

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AMMONIUM REMOVAL FROM AQUEOUS SOLUTIONS USING BANANA LEAVES MODIFIED BIOCHAR

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Abstract

Citizens worldwide are concerned about rampant water pollution as well as the application of the bioeconomy regarding reusing residual biomass from large-scale agricultural crops.

An approximate 128.78 million tons of banana were produced globally in 2019 according to data by the Food and Agriculture Organization of the United Nations. The banana's crop constitutes a significant source of economic growth, income, food security and nutrition of many developing countries, is consumed worldwide because of its availability throughout the year.

Biochar is a bioproduct of the pyrolysis of residual biomass and also is a low cost and environmental-friendly material that has a huge potential for removing pollutants from wastewater. This present work focuses on ammonium removal from water using biochar produced from banana leaves. First, the temperature to reach the maximum performance of the biochar concerning biomass through weight difference between the initial biomass and the obtained biochar was determined. After that, the ammonium efficiency removal was analysed through the batch studies with modification of pH, initial concentration of ammonium, the dose of biochar and contact time. The kinetic studies showed the adsorption isotherm data fitted well with Langmuir model. Meanwhile, the kinetics followed pseudo-second order model.

The results obtained in this study show biochar from banana leaves as an excellent material to remove ammonium from aqueous solutions.

Keywords: biochar, wastewater treatment, adsorption, ammonium removal, sustainability.

ENANTIOSELECTIVE SEPARATIONS WITH HIGH- AND ULTRAHIGH-PERFORMANCE CHIRAL LIQUID CHROMATOGRAPHY STATIONARY PHASES

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Abstract

High-performance liquid chromatography (HPLC) is a widely used technique. It can be applied for both preparative and analytical chromatography, for example in the food industry, bioanalytics, pharmaceutical industry, or environmental analysis. One important application area of HPLC is the so-called chiral separations; i.e. for the efficient separations of chiral compounds, techniques based on liquid chromatography employing chiral stationary phases (CSPs) are the most frequently applied solutions nowadays. The intense development of achiral stationary phases observed in the last decades resulted in a striking evolution of CSPs; reducing the physical dimensions of the HPLC columns CSPs with significantly higher theoretical plates have become commercially available. The full potential of these high-efficiency columns can only be exploited on chromatographic hardware specialized to ensure elevated pressures and low dead volumes, i.e. on ultrahigh-performance liquid chromatography, UHPLC.

The main technical difference between the two systems is the backpressure limit. Since the UHPLC systems can operate up to 1500 bars, smaller particles in the column can be employed, with lower column diameters, and shorter retention times, better resolutions and peak capacities can be achieved.

Macrocyclic glycopeptides are a relatively new type of selectors, but they are widely used nowadays in chiral separations. They can have multiple groups (hydroxyl, amino, carboxylic, etc.) that can interact with the sample molecules and can also contain multiple aromatic groups. Because of this wide range of possible interactions, macrocyclic glycopeptide-based columns are able to separate a lot of chiral components. For our experiments, we used teicoplanin and teicoplanin-aglycon as selectors.

We studied the enantiomeric separation of some β^2 -amino acids by performing experiments in an HPLC and a UHPLC system in a comparative manner.

Acknowledgments

This work was supported by the ÚNKP-21-3 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund, and the National Research, Development, and Innovation Office NKFIA through project grant K137607.

HOW CAN THE INTEGRATION OF ENVIRONMENTAL CONSIDERATIONS INTO CONSTRUCTION DECISIONS HELP?

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Abstract

Buildings around the world (their design, maintenance and disposal) contribute significantly to global warming. It is no coincidence that achieving the sustainable operation of buildings is a priority objective worldwide, not only in terms of the choice of materials but also in terms of achieving the best possible technological solutions. The aim of the project is to deliver the highest possible quality results in a comprehensible way to all audiences in any way connected with the building industry: interested parties, renovators, builders, architects, contractors, etc.

Introduction



Source: © 2018 2030, Inc. / Architecture 2030. All Rights Reserved. Data Sources: UN Environment Global Status Report 2017, EA International Energy Outbook 2017 The buildings (design, maintenance and end of life) contribute significantly to global warming, accounting for almost 30% of total greenhouse gas emissions, according to a recent study. This includes insulation, heating and cooling of buildings, but since everything is connected to everything else, it is in fact every component of it.

This paper shows the results of the Spread if Innovation Solution for Sustainable Construction project, what runs at the Adult Learning ERASMUS +-Strategic Partnership program. It is coordinated by LCA Center Association; from 2019.10.01 to 31/12/2021 and there four partners are working together, an Italian innovation spin-off company, Ecoinovazione srl., a Finnish software developer, BIONOVA ltd., as well as the EMI Nonprofit Ltd.



The main goal is to create awareness of ordinary people to learn about the importance of sustainability in terms of construction and prior to weigh on the market building materials and their properties. This helps if you are the life-cycle approach presented extraction of the environmental impacts of housing and building / apartment maintenance of building materials in the manufacture of building materials, materials beyond the integration from the end of the building's life cycle, i.e. the dismantling or demolition re-use. Increasing environmental awareness also comes to the fore in the project, because through it, indirectly facilitated by a move towards sustainability criteria within the building materials manufacturers, designers and contractors.

The researches began with a questionnaire survey, which was designed to get an idea of what you know about the public about sustainability, how to understand certain concepts and provide what information, and formulate what is required by your Web Application or related to manual. Answers about governing customize the pre-project tasks. In general, about the man in the street it expects housing to be efficient, to be comfortable, casual, but we are less sure how environmentally friendly solution can be achieved. Therefore, the established application and preparation of the manual was an important input.

The project produced two main outputs:

• One of them a Handbook and its annexes, which include important information about the built-in, redecorating life-cycle approach. It was written in four languages, was completed in pdf format. The English version is now available on the website (howtobuildgreen.eu).



• Other one is a web application, which includes the main technical characteristics and environmental data, and some market information in a variety of building materials (CFP or energy content, EPD). In addition, it is preparing a guide to aid the construction or renovation of a sustainable, environmentally conscious, or smart house through practical councils. We support the man on the street sensitization for use in education, environmental problems developed for the project elements.

27th International Symposium on Analytical and Environmental Problems

	Baseline CO2	e 402 kg/m² GFA Optimized	i CO ₂ e - kg/m² GFA	Carbon change -% / - ton	nes CO2e Assumptions	Select groupings
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	Choose types of constructions you wish	h to use, and adjust the materials used	In them as desired. You can	also save the adjusted data to a di	esign.	
Login	+ Foundation		1000 m ²	26 th	6.5%	
Remember me	+ Frost Insulation		83 m	0.92 tn	0.23%	
E English Français Deutsch Español Summi Norsk	+ Cleanliness layer		333 m ²	4.4 tn 1.1%	1.1%	
	+ Ground slabs		333 m ³	20 tn	4.9%	
Magyar	+ Floor slabs		667 m ³	48 th	125	

In the project, we examined different materials, structures and finished buildings proposed by architects from an environmental and economic point of view. The analysis of alternatives helps an external party to understand which options are better and more advantageous, and can help in future choices.

This paper shows the LCA and LCC results of different solutions, especially the renovation of Kadar cubic.



Environmental life cycle assessment provides an opportunity to understand the environmental impacts of our acts, processes, products, etc. In this work, an excellent team of experts has worked together to provide high quality, and the same time understandable insights for construction decisions. The team hopes that its work will be useful for all.

PALLADIUM RECOVERY BY ADSORPTION ONTO IONIC LIQUID-IMPREGNATED LAYERED DOUBLE HYDROXIDES

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Abstract

Palladium (Pd) is part of the platinum group metals (PGMs) and represents a "critical" raw material with economic importance. In our days, the secondary sources with Pd content are called "the urban mines of precious metals". Therefore, a lot of researchers focused their attention to the removal and recover studies of Pd ions from secondary sources. Regarding the recover process from aqueous solutions a lot of methods have been studied, among which adsorption process gained a special attention due to its well know advantages.

In this context, the present paper aims at the synthesis and characterization of Mg_3Al -LDH and its functionalization with IL(methyl trialkyl ammonium chloride) in order to obtain adsorbent materials with high efficiency in the removal process of Pd ions from aqueous solutions.

Introduction

It is well known that the sorption processes are more appropriate for the recovery of metal ions from diluted solutions. The challenge in the adsorption process is represented by the development of new efficient adsorbent materials. An effective adsorbent material must meet the following requirements: excellent selectivity, high adsorption kinetics and capacity, thermal and chemical stability, and ease of synthesis. These properties are fulfilled by layered double hydroxides (LDH). [1,2] For more than a decade, studies have been carried out in the functionalization of different solid supports with different functional groups to increase the adsorption capacity and the selectivity of these materials. To this end, the researchers' attention was focused on the use of ionic liquids (IL) in the processes of functionalization of solid supports. The reason for such interest comes from the almost unlimited possibilities of tunability / design of new ionic liquids for specific applications and needs, insignificant volatility, high thermal stability, and wide ionic and electrochemical conductivity. [3,4] Therefore, immobilization of IL in suitable solid supports is a solution for the removal of metal ions from aqueous solutions since the advantages of IL are combined with the properties of LDH.

Experimental

Mg₃Al-LDH was synthesized using the co-precipitation method at low-supersaturation. The functionalization of Mg₃Al-LDH with the same quantity of the studied ionic liquid (IL- methyl trialkyl – ammonium chloride) was realized through co-precipitation (sample named Mg₃Al-IL) and through ultrasonication (sample named Mg₃Al-ILUS). The obtained adsorbents were characterized by X-ray diffraction (RX), Fourier-transform IR (FTIR) spectroscopy, scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). The obtained adsorbent materials were used in the recovery process of Pd ions from aqueous solutions. The adsorption processes were conducted in batch mode.

Results and discussion

The XRD patterns recorded for the synthesised materials and functionalized with methyl trialkyl ammonium chloride are presented in Figure 1.



Figure 1. XRD patterns of the studied materials

The results of the characterization proved the fact that through ultrasonication, is assured a uniform distribution of the IL onto the surface of the LDH, while via co-synthesis is obtained an impregnation of the IL between the LDH layers, which will bring a higher stability of the obtained material. Palladium adsorption studies onto the studied materials were carried out in batch mode. The efficiency of the palladium adsorption onto the studied materials was determined by establishing the dependence of their adsorption capacity on the contact time, initial concentration of Pd (II) ions in the solution and temperature. The adsorption data were discussed from the kinetic and equilibrium point of view. The adsorption experimental data indicate that the adsorption process unfolds quickly, the equilibrium between Pd(II) ions and the studied adsorbent materials being achieved in 60 min. It is obvious that the functionalization of the Mg₃Al with methyl trialkyl ammonium chloride led to a an increase of approximately 50% of the maximum adsorption capacity of the studied materials in the recovery process of Pd(II) ions from aqueous solutions. The recovery degree of Pd(II) ions increased from 75.6% for raw Mg₃Al to over 95% for the functionalized materials. Data presented show that the kinetics of Pd(II) removal by adsorption on the studied material is described by a pseudosecond-order expression, because the correlation coefficient is very close to 1 and the theoretically predicted equilibrium sorption capacity is close to the value experimentally determined. Langmuir isotherm studies were conducted in order to investigate the maximum adsorption capacity and the affinity of the studied material towards Pd(II).

The Langmuir isotherm describe better the adsorption process of Pd(II) ions onto the studied material, comparted with other isotherm models due to the values of the obtained correlation coefficient which are close to unity. Moreover, the maximum adsorption capacity of the studied material obtained from the Langmuir plot is very close to that obtained experimentally.

Conclusions

It was observed that the presence of IL onto the LDH structure double the maximum adsorption capacity of the studied materials.

The maximum adsorption capacity of the studied materials increases in the order: $Mg_3Al \ll Mg_3Al$ -ILUS< Mg_3Al -IL. Comparing the maximum adsorption capacities developed by the layered double hydroxide with or without functionalization with methyl trialkyl ammonium chloride, in the process of Pd(II) recovering from the aqueous solution, with the adsorption capacities developed by other adsorbent materials reported in the literature, it can be concluded that the layered double hydroxide Mg_3Al is an efficient adsorbent, especially if it is functionalized with the studied IL.

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ECOTOXICOLOGICAL APPROACH OF SURFACTANTS TREATED BY IONIZING RADIATION

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Abstract

Removing surfactants from liquid effluents has been a fundamental issue since they are found in several industrial effluents, hospitals, restaurants, and even homes. When disposed into aquatic environmental, surfactants can implicate in significant changes, including several adverse effects to aquatic organisms. The present study was developed to assess the ecotoxicological effects of surfactants (anionic and nonionic) to aquatic organisms *Vibrio fischeri* bacteria, *Daphnia similis* crustacean. The ionizing radiation (Electron Beam Irradiation - EBI) was applied as a possible technology for the treatment of these contaminants in order to reduce acute toxic effects. The obtained data showed that the surfactants were toxic for both organisms, and the nonionic were more toxic than anionic. EB irradiation reduced surfactants toxicity by 40% acute toxic effects for nonionic surfactant and more than 70% for anionic, for both exposed organisms classes.

Introduction

The surfactant market is estimated to reach US\$ 39.86 billion by 2021, an increase of 5.4% between 2016 and 2021. Surfactants are the single most important ingredients in laundry and household cleaning products, comprising from 15 to 40% of formulation of these products. Furthermore, they are used in several industrial processes, such as in the textile, pharmaceutical, tannery, paper, chemistry industries [1,2].

Despite the importance of surfactants in daily life and industry processes, they are one of the main hazardous pollutants in wastewater and water bodies. Beyond the visual aspect of foam, the impacts of detergents on rivers are very critical. Practically the entire biota can be affected, with a significant loss of biodiversity. The biodegradation capacity of the water body is reduced due to the depreciation of the dissolved oxygen level in water. As a consequence of surfactants residues we have also the flowering of algae, which often releases toxins into the water bodies. Previous studies have been reported toxic effects of surfactants to different aquatic organisms including algae, daphnids, and bacteria, with data of effect concentrations (EC) lower than 0.5 mg L⁻¹ to *Vibrio fischeri* bacteria, for example [3,4]. Moreover, these contaminants can interfere in energy metabolism and transport of nutrients and oxygen; compromising the enzymatic activities and normal physiological function of organisms. Linear alkylbenzene sulphonate (LAS) in liver and internal organs of rainbow trout showed rapid absorption into systemic circulation [5,6].

Important concentrations of surfactants have been detected in surface waters and effluents in different regions of the world. For anionic surfactants: in Brazil, it was detected in surface waters concentrations between 14 and 155 μ g L⁻¹; in India, the levels in surface and ground water system are ranging from 2 to 62 μ g L⁻¹; in Nile river (Egypt) in surface water ranged between 0.07–2.45 mg L⁻¹ [7,8,9].

Regarding surfactants characterization, analytical and assessment techniques to achieve effective identification and quantification of several surfactants in different environmental matrices have been developed, with appropriate extraction, purification, and preconcentration. In this sense, several techniques such as spectrophotometry, titrimetry, and chromatography have been used [10,11].

Due the capability to specifically identify surfactant types, chromatographic techniques (mainly HPLC-MS and GC/MS) are the most common methods usually adopted to analyse various surfactants (anionic, cationic, non-ionic, amphiprotic, and semi-polar surfactant groups) and their degraded products, mainly when the surfactant is a component of an environmental matrix. Although relatively simpler techniques, such as spectrophotometric, can be applied if the surfactant is present as a single component [11].

The chemical characteristics, the low biodegradation, high toxicity of surfactants, confirm the needs for combined treatment processes for better degradability and in this regard, a possible technology may be ionizing radiation. The electron beam irradiation has been applied in several environmental matrices, for the treatment of industrial and domestic wastewater and hazardous pollutants [12,13].

In this context, the aim of the present study was to assess the acute toxicity of surfactants in aquatic organisms from distinct trophic levels, as well as electron beam treatment efficiency for toxicity reduction.

Experimental

Samples

The present study evaluated the surfactants nonionic (Alkyl aryl ethoxylated and aromatic sulphonate) and anionic (Sulphonated Dodecylbenzene) in an aqueous solution. The maximum concentration evaluated in acute assays was 1 g L^{-1} .

Electron beam irradiation

The liquid effluents were irradiated using a Dynamitron Electron Beam Accelerator and applying the following parameters: 1.4 MeV, fixed energy, batch system, conveyor speed of 6.72 m min–1 and variable electric current according to the required doses. The samples were distributed in pyrex vessels, ensuring 4 mm sample thickness, and the vessels were protected by plastic wraps. The samples were irradiated at 2.5 kGy dose.

Acute toxicity assays

Acute toxicity assays with *Daphnia similis* were carried out in accordance with NBR 12713: 2016 [14]. A minimum of five concentrations and a negative control with natural water (the same used in the *D. similis* culture) were evaluated per assay. Twenty organisms per concentration, at least 6–24 h, were exposed to the samples for 48 h. The observed effect after the exposure period was immobility. The results were expressed as EC50, the median concentration to observe an effect for 50% of all exposed organisms. Statistics were applied according to the standard methods recommendation, obtained by the Trimmed Spearman-Karber method.

The assays with *Vibrio fischeri* bacteria were performed in Microtox®, M-500, Microbics system. The bacteria luminescence was analyzed before and after 15 min of exposure to the samples; toxicity was evidenced by loss of luminescence. The assays were performed in triplicate, with a minimum of four concentrations, with a dilution factor of 2. The negative control with diluent solution (NaCl 2%) and a positive control with phenol were also performed. The methodology was in accordance with Brazilian Technical Standard Methods NBR

15411:2012 [15]. The EC50 values were obtained from a statistical procedure based on the gamma effect (γ) and the sample concentration (C%), using linear regression.

For both data, sample toxicity removal (%) after EBI treatment as calculated from EC 50 values, transformed to toxicity unit (UT), before and after irradiation.

Results and discussion

Acute toxic effects and EBI treatment values are reported in Table 1, Figure 1 and 2, respectively.

Table 1. Acute toxicity (EC50 values) and confidence limits (CL) of anionic and nonionic surfactants samples.

Aquatic organism	Surfactant	EC50 (mg L ⁻¹)	CL 95% (mg L ⁻¹)
D. similis	Anionic	19.7	(15.8-24.6)
	Nonionic	11.0	(9.2-13.2)
V. fischeri	Anionic	64.4	(23.7-74.6)
	Nonionic	2.3	(0.6-8.0)



Figure 1. Acute toxicity (EC50% values) of anionic surfactant versus applied EBI dose.



Figure 2. Acute toxicity (EC50% values) of nonionic surfactant versus applied EBI dose.

The acute effects data obtained herein showed that the surfactants were toxic for both organisms, which nonionic are more toxic than anionic, with EC50 lower than 11 mg L^{-1} (Table 1).

Previous studies reported acute toxic effects of surfactants to different aquatic organisms including algae, daphnids, and bacteria. For *V. fischeri*, were described values of EC50 = 0.35 mg L^{-1} (sodium dodecyl sulfate) and 1.92 (amine-oxide-based surfactant AO-R12) [3,4]. For several algae species exposed to cationic surfactants, median growth inhibition concentration ranged between 0.55-10.6 mg L⁻¹, with deleterious effects for photosynthetic activity [16]. While exposure to fish (*Clarias gariepinus*) LC50 (mg L⁻¹) values for anionic surfactants were 10.57-15.16 and for nonionic between 16.88-22.61 [17].

Regarding the results obtained after the EBI treatment (Figure 1 and 2), considering 2.5 kGy as a suitable dose for acute toxicity removal, about 40% acute toxicity removal were obtained for nonionic surfactant for both organisms. While the reduction was better for anionic, approximately 70% removal of acute effects for *D. similis* and 80% for *V. fischeri*.

Conclusion

The surfactants are critical issues for aquatic environmental and the present study showed that they were very toxic to both organisms. The proposal of this study was also to evaluate the toxicity reduction of the samples after treatment by electron beam. This technology has been obtaining promising results in the treatment of several organic compounds. In fact, in this study, the electron beam technology was effective in reducing the toxicity of the surfactants, even that the low, the effects reduction was considerable.

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NOVEL IRON COMPLEXES WITH GLYOXIMES, SCHIFF BASES AND BORIC ACID DERIVATIVES: SYNTHESIS, PHYSICO-CHEMICAL ANALYSIS AND BIOLOGICAL STUDY

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Abstract

Iron(II) clathrochelate complexes obtained with glyoximes are macrobicyclic ligand systems, which completely encapsulate the metal ion, and are formed under mild conditions with high yields [1]. In particular, the riblike-functionalized clatrochelates both with the inherent and with the terminal closo-borate substituents synthesized recently have been proposed as new radiopharmaceuticals for boron neutron capture therapy of cancer [2].

In our research work new iron(II) complexes were synthesized with α -glyoximes, boric acid derivatives, amines, Schiff bases, such as $[Fe(Me-Pr-Glyox)_3(BO-Et)_2]$, $[Fe(Et-Bu-Glyox)_3(BO-R)_2]$ (R = methyl, propyl, butyl), $[Fe(phenyl-Me-GlyoxH)_2(amine)_2]$, $[Fe(Et-Bu-GlyoxH)_2(amine)_2]$, $[Fe(2-heptanone)_2(en)(amine)_2]$, where GlyoxH, Glyox = mono- or bi-deprotonated glyoxime, en = ethylenediamine and the used amines: dibutylamine, 3-picoline, 4-aminopyridine, 6-amino-3-picoline, 3-amino-1-propanol, imidazole, 2-aminopyrimidine, 3-methylpiperidine, 3-amino-1H-1,2,4-triazole. For preparation iron^{II}-sulfate was dissolved in water and mixed with alcoholic solution of the glyoxime, then the corresponding amines and the other complexing agents were added. The mixture so obtained was refluxed under inert atmosphere.

The molecular structures of our products were studied by IR, Mössbauer and UV–VIS spectroscopies, mass spectrometry (MS) and thermoanalytical measurements (TG-DTG-DTA). The biological activity, like antimicrobial effect, was studied for a few bacteria.

Introduction

The unique properties of a metal ion encapsulated in the cage of a macropolycyclic ligand and isolated from the influence of external factors have allowed the use of clathrochelates as models of important biological systems, electron carriers, and catalysts of photochemical and redox processes [3].

Several iron chelates have been reported for application in the treatment of thalassaemia, other transfusion-dependent diseases, and also used as MRI contrast agents. Other iron complexes are also known for their antibacterial, antifungal and biomimetic activities [4].

Schiff bases play an important role in inorganic chemistry as they easily form stable complexes with most transition metal ions. These days, bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve

as models for biologically important species. The remarkable biological activity of the acid hydrazide (R–CO–NH–NH₂) class of Schiff base, their corresponding aroyl hydrazones (R–CO–NH–N=CH–R) and the dependence of their mode of chelation with transition metal ions present in the living systems have been of significant interest. The coordination compounds of aroyl hydrazones have been reported to act as enzyme inhibitors and are useful due to their pharmacological applications [5].

In this paper we report the synthesis, characterization and biological evaluation of novel iron complexes with glyoxymes, Schiff bases and boric acid derivatives.

Experimental

<u>Used materials</u>: FeSO₄·7H₂O, Me-Pr-GlyoxH₂, Ph-Me-GlyoxH₂, Et-Bu-GlyoxH₂, boric acid, borax, ascorbic acid, 2-heptanone, ethylenediamine, dibutylamine, 3-picoline, 4-aminopyridine, 6-amino-3-picoline, 3-amino-1-propanol, imidazole, 2-aminopyrimidine, 3-methylpiperidine, 3-amino-1*H*-1,2,4-triazole, MeOH, EtOH, *n*-PrOH, *n*-BuOH. Methods:

- Synthesis of [Fe(Glyox)₃(BO-R)₂] type complexes

 $0.0075 \text{ mol Me-Pr-GlyoxH}_2$ or Et-Bu-GlyoxH}_2 was dissolved in 20 ml MeOH or EtOH or PrOH or *n*-BuOH, then this solution was added to an aqueous solution of 0.0025 mol (0.7 g) FeSO₄ and 0.4 g ascorbic acid dissolved in 25 ml water. The role of ascorbic acid is to prevent the oxidation of Fe^{II} to Fe^{III}. Afterwards 0.0075 mol (0.46 g) boric acid dissolved in 15 ml H₂O was added. The mixture was refluxed for 15 min under inert atmosphere, and then 0.00375 mol (1.4 g) borax dissolved in 15 ml distilled water and 55 ml of the corresponding alcohol were added. The obtained solution was heated for 2–3 hours on a water bath, under inert atmosphere. After cooling the crystalline complexes were filtered off, washed with the used alcohol and diethyl ether, then dried in air. A typical reaction is shown below:



- Synthesis of [Fe(GlyoxH)₂(amine)₂] type complexes

0.005 mol phenyl-Me-GlyoxH₂ or Et-Bu-GlyoxH₂ was dissolved in 20 ml EtOH and this solution was added to the aqueous solution of 0.001 mol (0.3 g) FeSO₄ and 0.4 g ascorbic acid dissolved in 10 ml water. After that 0.002 mol amine (dibutylamine, 3-picoline, 4-aminopyridine, 6-amino-3-picoline, 3-amino-1-propanol, imidazole, 2-aminopyrimidine, 3-methylpiperidine) dissolved in 5 ml EtOH was added. The obtained solution was heated for 2–3 hours on a water bath under inert atmosphere. The filtered crystalline complexes were washed with EtOH–water mixture (1:1) and diethyl ether. A typical reaction as an example:



- Synthesis of [Fe(2-heptanone)₂(en)(amine)₂] type complexes

0.005 mol 2-heptanone (0.7 ml) and 0.0025 mol (0.19 ml) ethylenediamine were dissolved in 5 ml EtOH, then refluxed for 1–2 hours. The resulting colored solution was added to the aqueous solution of 0.0025 mol (0.7 g) FeSO₄ and 0.4 g ascorbic acid dissolved in 15 ml water. At last 0.005 mol amine (imidazole, 3-amino-1*H*-1,2,4-triazole) dissolved in 10 ml EtOH was added. The obtained mixture was refluxed in a water bath for 2–3 hours. After cooling the crystalline complexes were filtered off, washed with EtOH–water mixture (1:1) and diethyl ether. Finally the crystalline complexes were dried in air. A typical reaction:



Results and discussion

Microscopic characterization and yields of the prepared complexes are presented in Table 1.

Table 1. Microscopic characterization, calculated molecular weights and yields of the prepared complexes.

Nr	Compound	Calc. mol.	Yield	Microscopic
111.	Compound	weight	(%)	characterization
1.	[Fe(Me-Pr-Glyox) ₃ (BO-Et) ₂]	594.06	76	Brown, small triangle-based prisms (microcrystals)
2.	[Fe(Et-Bu-Glyox) ₃ (BOMe) ₂]	650.16	10	Greenish-brown triangle-based prisms (microcrystals)
3.	[Fe(Et-Bu-Glyox) ₃ (BO–n-propyl) ₂]	706.27	84	Black triangle-based prisms
4.	[Fe(Et-Bu-Glyox) ₃ (BO-n-butyl) ₂]	734.33	95	Dark brown triangle-based prisms
5.	[Fe(phenyl-Me-GlyoxH) ₂]	410.21	45	Reddish-brown irregular microcrystals
6.	[Fe(phenyl-Me-GlyoxH) ₂ ((n-Bu) ₂ NH) ₂]	668.70	42	Reddish-brown triangle-based prisms
7.	[Fe(phenyl-Me-GlyoxH) ₂ (3-picoline) ₂]	596.46	45	Reddish-brown triangle-based prisms (microcrystals)
8.	[Fe(phenyl-Me-GlyoxH) ₂ (4-amino-pyridine) ₂]	598.44	32	Reddish-brown triangle-based prisms
9.	[Fe(phenyl-Me-GlyoxH) ₂ (6-amino-3-picoline) ₂]	626.49	70	Reddish-brown triangle-based prisms
10.	[Fe(phenyl-Me-GlyoxH) ₂ (3-amino-1-propanol) ₂]	560,43	90	Purple-brown, small triangle- based prisms (microcrystals)
11.	[Fe(Et-Bu-GlyoxH) ₂ ((n-Bu) ₂ NH) ₂]	656.77	40	Reddish-brown irregular microcrystals
12.	[Fe(Et-Bu-GlyoxH) ₂ (6-amino-3-picoline) ₂]	614.57	96	Brown triangle-based prisms (microcrystals)
13.	[Fe(Et-Bu-GlyoxH) ₂ (3-amino-1-propanol) ₂]	548.50	48	Brown triangle-based prisms
14.	[Fe(Et-Bu-GlyoxH) ₂ (imidazole) ₂]	534.44	65	Reddish-brown triangle-based prisms (microcrystals)
15.	[Fe(Et-Bu-GlyoxH) ₂ (2-amino-pyrimidine) ₂]	588.49	89	Reddish-brown triangle-based prisms (microcrystals)
16.	[Fe(Et-Bu-GlyoxH) ₂ (3-Me-piperidine) ₂]	596.63	34	Reddish-brown triangle-based prisms
17.	[Fe(2-heptanone) ₂ (en) (imidazole) ₂]	442.43	48	Brown triangle-based prisms (microcrystals)
18.	[Fe(2-heptanone) ₂ (en) (3-amino-1H-1,2,4-triazole) ₂]	474.43	23	Dark brown triangle-based prisms

Infrared spectroscopic study

The mid-IR spectra were recorded with a Bruker Alpha FTIR spectrometer (Platinum single reflection diamond ATR) and Bruker Vector 22 at room temperature, in the wavenumber range of $4000-400 \text{ cm}^{-1}$. The samples were measured in solid state (in powder form) or in KBr pellets, respectively. The data of the most characteristic IR bands for the selected complexes are presented in Table 2.

Comp. cm ⁻¹	1	2	3	4	5	6	11	14	17	18
V О–Н	-	-	-	-	3402 w	3281 w	3721 w	3721 w	-	-
VN-H	3184 s	3447 s	3385 s	3447 s	3227 m	3175 w	3185 w	3553 w	3393 vs	3354 vs
νс−н	2956 w	2928 w	-	2928 m	3057 w	2956 w	2918 m	2917 m	2995 m	2781 w
VC=C	-	1684 w	1734 m	1747 vs	1678 m	1643 s	1646 vs	1646 vs	-	1647 vs
VC=N	1400 vs	1636 m	1636 s	1647 s	1645 m	1541 m	1552 s	1553 s	1636 vs	1553 s
бсн2	-	1420 m	1420 m	1456 m	1414 s	1440 s	1451 s	1451 m	-	1454 w
бснз	-	1385 s	1385 s	1383 m	1365 m	1372 s	1392 m	1391 m	1396 m	1398 w
VN-N	-	-	-	-	-	-	-	-	-	1124 vs
VN-O	-	-	-	1115 vs	1195 m	1230 s	1173 s	1175 s	-	-
V N–ОН	-	-	-	-	1015 s	1106 vs	1103 vs	1102 s	-	-
VB-O	1185 vs	1140 vs	1111 vs	1016 vs	-	-	-	-	-	-
то-н	1044 m	995 m	945 s	934 s	972 vs	947 vs	963 vs	967 s	1069 s	984 s
үс-н	711 vs	621 s	617 s	619 s	708 vs	694 vs	734 s	734 s	627 s	615 s
VFe–N	543 vs	-	-	-	483 s	557 s 426 vs	516 s 418 vs	530 s 418 vs	-	-

Table 2. IR data of the selected complexes.

(Abbreviations: vs = very strong, s = strong, m = medium, w = weak)

The most important bands for the characterization of complexes are $v_{C=N}$ (1400 – 1647 cm⁻¹) and v_{Fe-N} (418 – 557 cm⁻¹). If we compare the influence on the nature of the glyoxime ligand (aliphatic or aromatic), we can observe the displacement of $v_{C=N}$ band to higher values and the displacement of v_{Fe-N} band to lower values in case of aliphatic ligand, which are in accordance with the electronic effects.

Mass spectrometry

Mass spectra of the samples were recorded using electrospray ionization (ESI). In the spectra we could detect the molecular ions and some decomposition fragments.

<u>Mössbauer spectroscopy</u>

The Mössbauer spectra were recorded at room temperature (295 K) and liquid nitrogen temperature (78 K) with Wissel type Mössbauer spectrometer in constant acceleration mode and in transmission geometry.

The Mössbauer spectroscopic measurements indicate the oxidation and spin state of Fe, and also the purity of the complexes. In case of aromatic ligands the high spin Fe^{III} oxidation state

is observed due to the electron attraction of the ligand, however, in case of aliphatic ligands we obtain low spin Fe^{II}.

Thermoanalytical measurements (TG-DTG-DTA)

Thermal measurements were performed with a 951 TG and 910 DSC calorimeter (DuPont Instruments), in Ar or N_2 at a heating rate of 10 Kmin⁻¹ (sample mass of 4–10 mg).

The thermal stability of complexes is limited within the temperature range of 50-100 °C. In the case of [Fe(Glyox)₃(BO-R)₂] type complexes the first decomposition step belongs to leaving RO group, until 170 °C, then the BO_x part is lost until 300 °C. Subsequently, the decomposition of the glyoxime unit takes place, which is accompanied by big exothermic peaks. This behavior can be explained with the presence of oxygen in the molecule. The process ends at 700 °C. In the case of [Fe(GlyoxH)₂(amine)₂] type complexes the first step of the decomposition mechanism is the loss of the amino group between 50-200 °C, then the glyoxime units leave. The end of the process is at 500 °C. The decomposition of [Fe(2-heptanone)₂(en)(amine)₂] type complexes begins with leaving of the amino groups until 300 °C, then the heptanone unit leaves. Finally the N-CH₂-CH₂-N unit is lost between 50-700 °C. The general mechanism for decomposition is as follows:

$$\begin{split} [Fe(Glyox)_3(BO-R)_2] &\rightarrow [Fe(Glyox)_3(B)_2] \rightarrow [Fe(Glyox)_3] \rightarrow Fe_2O_3 \\ [Fe(GlyoxH)_2(amine)_2] \rightarrow [Fe(GlyoxH)_2(amine)] \rightarrow [Fe(GlyoxH)_2] \rightarrow [Fe(GlyoxH)_2] \rightarrow \\ &\rightarrow Fe_2O_3 \\ [Fe(2-heptanone)_2(en)(amine)_2] \rightarrow [Fe(2-heptanone)_2(en)(amine)] \rightarrow [Fe(2-heptanone)_2(en)] \rightarrow \\ &\rightarrow [Fe(2-heptanone)(en)] \rightarrow [Fe(en)] \rightarrow Fe_2O_3 \end{split}$$

UV-VIS spectroscopy

The electronic spectra were recorded with a Jasco V-670 Spectrophotometer in 10% EtOH/water solutions containing the substance in 10^{-4} mol/dm³ concentration. Using Sörensen buffer solutions the electronic spectra were also recorded as a function of pH, and then the acidity constants were calculated, too. The obtained values were between $1.2 \cdot 10^{-11} - 1.1 \cdot 10^{-10}$.

Biological study

The antimicrobial effects for two complexes: $[Fe(Et-Bu-Glyox)_3(BOMe)_2]$ and $[Fe(Et-Bu-Glyox)_3(BOPr)_2]$ were studied with Bacillus Subtilis Gram-positive and Escherichia Coli Gram-negative bacteria. The observation was made with the disk method. The complexes were dissolved in DMSO with 10 mmol/l concentration. In both cases antibacterial effect was not observed.

Conclusion

In this work new iron(II) complexes were synthesized and characterized with physico-chemical methods. Thermal decomposition mechanism was monitored with thermoanalytical measurements. Antibacterial activity was also investigated.

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METAL CONTENT OF SEASHELLS FROM BLACK SEA'S ROMANIAN COAST

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Abstract

The main metals from various species of seashells, commonly found on the Black Sea's Romanian coast, were determined by using microwave plasma atomic emission spectroscopy (MP-AES) and energy-dispersive X-ray spectroscopy (EDX). As expected, calcium was the main component, followed by magnesium, aluminum, iron and manganese.

Introduction

Although seashells are generally regarded as waste products with little commercial value, they can be chemically modified to give useful materials by using simple technologies. For example, they can find applications as support for growing biomass, for heavy metal decontamination and for hydroxyapatite fabrication [1].

Various authors studied the elemental distribution in marine organisms and sediments from the Black Sea coast. By using atomic absorption spectroscopy (AAS), Cadar et al. determined the heavy metal (Cd, Cu, Pb and Zn) concentrations in marine water, sediments and algae from the Romanian coast in 2017 and 2018 and found some contamination related to the harbor activities (Constanța and Mangalia) [2]. Heavy metals (Cd, Co, Cr, Hg, Ni, Pb, Sn) from tellina (*Donax trunculus*) on the Turkish coast were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and it was found that temperature changes affected the metal accumulation in the species [3]. Trace elements (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) were determined by ICP-OES in the Black Sea mussel (*Mytilus galloprovincialis*) and rapa whelks (*Rapana venosa*) from Bulgarian coast and they were found not to exceed the maximum residual levels prescribed for seafood [4]. Also, the chemical composition (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Ti, V, Zn) of *Rapana thomasiana* from the Romanian coast was studied with ICP-OES by Sereanu et al. in order to assess the degree of shells mineralization [5].

During our efforts to find practical applications for seashells as biomass support and hydroxyapatite precursors [6-8], we made some preliminary analysis regarding the elemental distribution in molluscan shells collected from the Black Sea's shore.

Experimental

Reagents (65% HNO₃, 28-30% NH₃) were analysis grade from Merck.

Raw seashells were collected in 2020 and at the beginning of 2021 from a Black Sea beach (43.819222 N, 28.589222 E) in the city of Mangalia, Romania (Figs. 1 and 2). They were dried naturally for seven days before further processing.



Figure 1. Seashell collection site in Mangalia, Romania



Figure 2. General aspect of the collection site

For MP-AES analyses, some of the most common types of seashell species found in the Black Sea were selected: *Mytilus galloprovincialis* (sample 1), *Mya arenaria* (sample 2) and *Cerastoderma edule* (sample 3). Dry seashells were washed using an ultrasonic bath and were carefully cleaned on the surfaces of the exoskeleton with a plastic brush. The shells were crushed by pressing them, after which particles smaller than 2 mm were separated using a sieve. These were dried at 115 °C for 20 minute after which 2.000 g of each sample was calcined at

550 °C for 10 h in alumina crucibles with a temperature increase of 5 °C/min. The crucibles are brought to room temperature and the samples are weighted, after which they are kept in a desiccator in the crucibles with concentrated HNO₃ until complete digestion. After dilution with double-distilled water, the samples were analyzed using an Agilent MP-AES 4100 spectrometer, with the following parameters for all samples and wavelengths: 0 degree viewing position, 120 kPa nebulizer pressure (nitrogen as carrier gas), 30 seconds sample uptake time, 15 seconds stabilization time, 10 seconds read time and 3 replicates for all measurements. All glassware was washed with 2% HNO₃ and rinsed with double-distilled water. Solid CaCO₃, Fe₂O₃ and metallic magnesium were dissolved in HNO₃ in order to prepare the three standard stock solutions (1000 ppm calcium, 1000 ppm iron and 100 ppm magnesium). Calibration used five levels (0.00, 0.80, 2.00, 10.00, 20.00 ppm for calcium and 0.00, 0.60, 1.50, 7.50, 15.00 ppm for magnesium and iron). For maximum sensibility, calibration and data recording used the top five most intense emission lines offered by the spectrometer software, leaving aside those lines that interfere with sodium and potassium (393.366, 396.847, 422.673, 430.253, 445.478 nm for calcium, 279.553, 280.271, 285.213, 383.829, 518.360 nm for magnesium and 259.940, 302.064, 358.119, 371.993, 385.991 nm for iron). Two dilutions were used for the three samples which were analyzed: for magnesium and iron, present in much lower quantities, the samples were 50 times more concentrated then those for calcium.

For EDX analyses, dry raw seashells were first sieved through a 1 cm mesh sieve. The larger fraction was kept on the sieve and the small foreign bodies were disregarded by blowing compressed air. The samples were then washed in the ultrasonic bath for half an hour, after which they were immersed in 6% sodium hypochlorite for 3 x 24 h to remove the outside organic matter. After this treatment, the samples were repeatedly washed with tap water and finally with double-distilled water. After drying in the oven at 80 °C, the samples were immersed in 1 M NaOH for 24 h in order to remove any organic residues from the surface of the exoskeletons [9]. They were again repeatedly washed with tap water and finally with double-distilled water, then they were dried at 80 °C. The dried samples were mechanically broken and the 4-7 mm fragments were isolated using a custom made sieving device. Heavy metals from seashells were concentrated for EDX analysis by using sulfide precipitation. In order to achieve this, the samples were digested by using 65% HNO₃ (200 mL of acid for 100 grams of seashells). The yellow suspension was boiled until the volume was reduced to half, during which the liquid became almost clear. The pH was adjusted to 10 by using ammonia and the mixture was diluted with double-distilled water until the volume was doubled. Sodium sulfide was added until the reaction with silver nitrate gave a positive result (0.3 mL of 0.1 mol L^{-1} Na₂S). The dark-green precipitate that formed was subjected to centrifugation and washing with double-distilled water (two cycles), after which it was dried in an oven at 105 °C and subjected to EDX analysis by using an accelerating voltage of 25 kV.

Results and discussion

In the case of MP-AES analyses, after inspecting the correlation coefficient (r) and calibration errors, the 422.673 nm line (r=0.99981) was selected for calcium and the 279.553 nm line (r=1.00000) for magnesium. By comparing the amount of calcium determined with MP-AES and the mass of the samples it was confirmed that the exoskeletons are made almost entirely of calcium carbonate, with only traces of other elements. The weight ratios between calcium and magnesium obtained for the three samples were 224.8:1 for the first sample, 1180.8:1 for the second sample and 1067.2:1 for the third sample, with a mean value of 824.3:1. Iron was below the detection limit of all monitored wavelengths and for all samples, even though the calibration correlation coefficient was very good (r=0.99997 for 259.940 nm and r=0.99999 for 385.991 nm) and the calibration errors were minimal (under 3.33%) for the selected wavelengths.





Figure 3. Peaks in EDX analysis of sulfide residue

Table 1.	Percentages	of each	element i	in EDX	analysis	of sulfide	residue

Element	Weight %	Atom %
O K	60.46	66.52
Mg K	9.41	6.81
СК	6.35	9.30
N K	5.93	7.46
Si K	5.90	3.70
Ca K	3.06	1.34
S K	2.73	1.50
Al K	2.57	1.68
P K	2.22	1.26
Fe K	1.23	0.39
Mn K	0.15	0.05
Total	100.00	100.00

In the EDX spectrum, the presence of C, N, O and P elements was observed, which may originate from the organic matter existing between the aragonite lamellae from the exoskeletons. Due to the fact that these elements were not quantitatively isolated in the analyzed sample, no conclusions can be drawn regarding their relative abundance in the shells. Sulfur can originate from both protein mass and sulfide added to precipitate heavy metals. Also, the residual calcium and magnesium salts originate from the incorporation in the mass of the precipitate. The largest amount of calcium and magnesium was removed during the heavy metal separation protocol. Finally, the ratio of the elements extracted quantitatively from the sample is Si : Al : Fe : Mn = 63.6 : 28.9 : 6.7 : 0.9 (atomic %) or Si : Al : Fe : Mn = 59.9 : 26.1 : 12.5 : 1.5 (mass %). The other elements are below the spectrometer detection limit.

Conclusion

EDX and MP-AES analyses revealed calcium to be by far the main metallic component, followed by traces of magnesium, aluminum, iron and manganese. Although some nonmetals, namely oxygen, carbon, nitrogen, silicon, sulfur and phosphorus were also identified in EDX analyses, only silicon can be quantified with the applied protocol.

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ACCURATE DETECTION OF SARS-CoV-2 MIGHT BE A CHALLENGE IN THE MOLECULAR BIOLOGY LABORATORY FOR RT-PCR FINAL RESULTS

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Abstract

The challenges we experience professionally always teach us to retreat, to document ourselves, to learn, to become better and to succeed in asserting ourselves in the fields we have trained and perfected throughout several years. This also happened in the pandemic times with Covid-19, when we had to document RT-PCR techniques in order to be able to detect at the molecular level the SARS-CoV-2 virus responsible for disturbing the world worldwide. Thus, in the molecular biology laboratory, analysts must make very important decisions about the final result of the RT-PCR test to determine – given several analytical criteria – whether the result is negative, positive or equivocal. There are situations where the RT-PCR equipment does not detect all the genes responsible for a clearly positive result, or when the cycle threshold (Ct) is higher than specified in the reagent insert kit, and then it is the analyst's duty to decide what is the final result of molecular test. This paper brings to the fore the involvement, duty and the art of researchers and specialists who must assume the final result of the RT-PCR test in the detection of the SARS-CoV-2 virus responsible for the global pandemic of Covid-19.

Introduction

We are in the midst of a COVID-19 pandemic, and in Romania at the moment, we are sad and terrified of the 4th wave of SARS-CoV-2 infection, when the infection rate exceeded 12 positive cases per 1000 inhabitants, and when hospitals have courtyards full of ambulances waiting for a place available in the hospital to transfer critically ill patients, and unfortunately, patients are also getting younger and younger.

We go back a little in time, in 2013, and we remember the onset of the viral disease with the virus SARS-CoV-1 (Severe Acute Respiratory Syndrome Coronavirus), which underwent mutations being named in 2018 MERS-CoV (Middle East Respiratory Syndrome Coronavirus), so that in 2019 we reach the SARS-CoV-2 virus (Severe Acute Respiratory Syndrome Coronavirus) [1, 2, 3].

Testing methods for the detection of SARS-CoV-2 virus are very important, because the accurate molecular result plays a very important role in the correct diagnosis and ideal treatment prescription, enabling the doctor to cure quickly and confident the patients [4, 5]. Thus, the rRT-PCR – the real time reverse transcription polymerase chain reaction is the molecular test mostly used in the detection of the nucleic acid of SARS-CoV-2 virus. This technique detects usually two or three of viral genes responsible for the infection, and one common *Coronaviridae* family. The SARS-CoV-2 virus is a beta coronavirus, single-stranded RNA, positive-sense, part of *Coronaviridae* family, and *Orthocoronavirinae* subfamily. From molecular point of view, the genome encodes some specific glycoproteins (spike – S, membrane – M, envelope – E, and nucleocapsid – N) which can be detected with PCR technique [6-10].
For the first time, the quantitative rRT-PCR technique for detection of SARS-CoV-2 virus was developed in Germany, at the Charité Institute of Virusology, and it was reported also by World Human Organization (WHO) last year (2020) on January 13. Followed this method there were subsequently reported others additional protocols which have as assay target three different specific genes: SARS-CoV-2 RNA-dependent-RNA-polymerase (RdRp), E and N gene [11]. For all RT-PCR techniques, for all protocols, there are necessary approves from Emergency Use Authorization (EUA) for reagents; for extraction, sequences and amplification; analytical sensitivity, specificity, and precision.

The results of RT-PCR are very important for the correct diagnosis, but also some others aspects have to be taken in consideration for the right diagnosis and the best treatment prognosis, such as: collection of nasopharyngeal secretions (swab type, sampling and collection method); transportation and preservation of the samples; blood tests (neutrophils, inflammatory biomarkers, lymphocytes); Ct – cycle threshold value; and if necessary the CT (computer tomographic) examination.

Sometimes the RT-PCR results have to be evaluated taking in consideration not only the final result, but also the detection of both two genes (common family *Coronaviridae* – E gene and also SARS-CoV-2 gene), Ct value for both genes, as well as the graphical shape of all detected genes. But for this it is the task of the analyst to decide if the final result is positive or negative, even the RT-PCR amplification equipment gives the final result "positive", "presumptive positive" or "equivocal". Our paper debates exactly this situation where the final result has to be decided having in view not only the result of the amplification equipment, but also some other aspects regarding the amplification information and the reagent insert kit specifications.

Experimental

The RT-PCR technique is a molecular method of detection of nucleic acid of SARS-CoV-2 virus in order to establish if the patient is infected or not! The protocol involves the swabsampling of the nasopharyngeal secretion, transportation and preservation of the samples, RT-PCR extraction and amplification. The analytical work was done in the Laboratory of Molecular biology from Banat's University of Agricultural Sciences and Veterinary Medicine "King Mihai the Ist of Romania" from Timisoara, Romania. The sampling preparation was performed in a biological safety cabinet class II (hood with biosecurity level 2), and the personnel wore protective equipment according to biosafety specifications. The extraction was performed with an automatic RT-PCR extractor 48 from Bioneer, and the amplification was performed with the RT-PCR Exicycler 96 from Bioneer.

We evaluated the RT-PCR results since 1st of January 2021 to 30th of September 2021 from the Laboratory of Molecular Biology from Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael the Ist of Romania" from Timisoara, Romania (BUASVMT). Thus, we performed a number of 2590 total tests, from which the RT-PCR equipment gave us 2440 negative results, 150 positive results, and 57 presumptive positive results – that represents 2.2% from total RT-PCR tests. This means that at least 2.2% (presumptive or inconclusive positive) from total RT-PCR results had to be re-evaluated by the analyst in the laboratory for setting the final RT-PCR result which was reported also to the Ministry of Health from Romania and also to the patients.

Results and discussion

For an accurate RT-PCR result, for each run we include a negative and a positive control, that is part of the insert reagent kit, and is absolutely necessary for an internal quality control. The result for each control run (negative and positive) has to be "valid". Also, each sample amplified

on the RT-PCR equipment contain the Bioneer's AccuPower PCR PreMix reagent and also internal control, and for all samples we have to have a valid result for internal control.

The results of the RT-PCR amplification can be clear-cut, negative or positive for SARS-CoV-2, with valid internal control, with non-detected gens of *Coronaviridae* family and SARS-CoV-2 specific gene – for negative result; and with Ct (cycle threshold) value for both E. and SARS-CoV-2 gene – for positive result.

The Ct value is very important and could be a very important specification, but not singular, in the right diagnosis and best treatment for the patient! The Ct – cycle threshold in RT-PCR is a positive reaction and response, being detected by accumulation of a fluorescent signal. Ct is the number of cycles required for the fluorescent signal to exceed the background level, and is inversely proportional to the amount of target nucleic acid from the sample. Thus, a high Ct value is correlated with a low viral RNA-SARS-CoV-2 load, and of course a lower risk of Covid-19 transmission [12]. The RT-PCR assay undergo up to 40 cycles for amplification, but the maximum value of Ct for positive results is set by the producer of the insert reagent kit. For Bioneer's AccuPower PCR reagent kit, the maximum value of Ct for positive results is lower than 33.

However, the RT-PCR equipment gives as a final result sometimes a "presumptive SARS-CoV-2 positive" result, that means that the result is equivocal, is not certain positive.

So, what we should do in this case? How we have to evaluate all the data and give a correct final result? In this case we have to evaluate not only the final result, but we have to check the detection of all genes, the values of Ct for E gene and SARS-CoV-2 gene, and also we can evaluate the graphical presentation of the amplification curves. Figure 5 presents a case of "presumptive SARS-CoV-2 positive", but because we can easily see that only the SARS-CoV-2 gene was detected and the Ct was 31.87, and the E gene was non-detected, we can give as a final result as negative for SARS-CoV-2 infection.

Based also on literature data and the specifications of the reagent kit producer, we can consider a result of suspect, inconclusive or weak positive with Ct values higher than 33, as indicating minimal amounts of target nucleic acid, in our case SARS-CoV-2-RNA could represent early or late infection, residual vaccine or even environmental contamination.

Other situations can be identified as a "positive test result from RT-PCR", with valid negative and positive control for presented run.

Even if the final reported result from RT-PCR after amplification is "positive test result", when we check the graphical representation we can observe that E gene is not detected, SARS-CoV-2 gene is detected – but the Ct value exceed the reagent kit recommendation (as maximum 33 value for positive result), and IPC (internal control is valid). So, in this case the final result reported to the patient is "negative for SARS-CoV-2 infection"!

There are many scientific experimental papers published about the Ct value regarding COVID-19, which try to elucidate and make clear the importance and the right evaluation and interpretation of the Ct value. Thus, Polese-Bonatto and her research team evaluated the Ct value for children and adults infected with SARS-CoV-2, using RT-PCR confirmed infection, using a method that detects three gene-targets, and the results were comparable between children and adults. On the other hand, Ct value is correlated with some hematological and blood biochemical parameters, such as: aspartate-aminotransferase (AST), neutrophils, and monocytes [13].

Other scientific reports presented clear conclusive data about strong correlations between Covid-19 and some blood biochemistry, coagulation tests, and inflammatory factors, such as: leucocytes, lymphocytes, neutrophils, platelet, hemoglobin, total protein, globulin, albumin, procalcitonin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), antistreptolysis O (ASO), D-dimer, fibrinogen, lactate dehydrogenase (LDH), interleukin-6 (IL-6), interleukin-2 receptor (IL-2R) [14-18].

There is another situation where the RT-PCR result is "invalid". In this case, first we have to evaluate if the IPC (internal control) is valid or not for that sample, if the negative and positive control for that run is valid, and also to check if there is any of the specific gene detected.

An example of this situation presents the final RT-PCR test result as "invalid", the IPC is also "invalid", and none of the E gene and SARS-CoV-2 gene is detected. In this case the final RT-PCR result is "Equivocal" that means that the test has to be performed again, and we have to repeat extraction and amplification to be sure for the final result.

More and more researchers try to make correlations between different blood tests results, computed tomography (CT) evaluation, radiographic evaluation (RX), and magnetic resonance imaging (MRI) evaluation, encephalography technique, just to find new and new findings which can in recent future to prevent and treat with success this pandemic disease of Covid-19!

Bairwa and his team found strong association with Covid-19 mortality of some hematological and biochemical markers. Thus, they evaluated 249 hospitalized patients infected with SARS-CoV-2 which presented elevated procalcitonin, C-reactive protein, AST, serum potassium, neutrophils count, RBC (red blood cells) count, and prothrombin time; while the lymphocyte count, oxygen saturation, partial oxygen pressure, alanine-aminotransferase (ALT), and lactate dehydrogenase decreased – most of the situations having strong association with mortality [19].

World Human Organization (WHO) Working Group on the Clinical Characterization and Management of Covid-19 infection publishes regular reports about the new findings regarding this new and pandemic disease published in Lancet – Infectious disease a clinical report where they underlined some endpoints used in clinical studies planned and done during Covid-19 outbreak and also clinical progression scale, presented a score of severity of disease from "uninfected" to "dead" of Covid-19 [20].

Having all this very complex data in our view, trying to bring new experimental proves and findings into the world attention, we are part of this scientific work, and, also with our experimental experience we come and add information that can help in this fight against the SARS-CoV-2 virus and help as much as we can for all people to return to their previous lives, to get through the disease easily or to get vaccinated and thus to develop antibodies that protect us in the near future.

Conclusion

RT-PCR technique is used with success for qualitative and quantitative detection of SARS-CoV-2 RNA viral infection and Covid-19 disease. The samples represents swab nasopharyngeal secretions and the molecular test involves the extraction of the RNA and the amplification on RT-PCR.

Most of the time the results from RT-PCR technique is very clear, is "positive SARS-CoV-2" or "negative", but sometimes the result could be "invalid" or "presumptive SARS-CoV-2 positive" – and in these situations the analyst from the laboratory, usually geneticist specialist (medical doctor, biologist, biochemist, or chemist) has to evaluate all data and information from the equipment and to give the final medical report.

The geneticist plays a critical role in the final decision of the final medical report and result of the RT-PCR test. But there are more characteristics that should be taken in view and should be evaluated. Because all these, the cooperation between the medical doctor that follow the medical state of the patient, the laboratory specialists, and the family doctor for the historical medical data for the patient in critical and very important.

Prevention, correct diagnosis, medical prognosis, and right treatment could be the difference between a medical success and the medical failure!

Not the last, very important is the pre-analytical phase (sampling the biological specimen - in our case the nasopharyngeal secretions), analytical and post-analytical phase plays all very important role in the great achievements and professional recognition!

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Poster Proceedings

ELECTROCOAGULATION REMOVAL OF HEAVY METALS FROM WASTEWATER GENERATED BY WASHING A SCREEN PRINTING PLATE

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Abstract

The possibility of applying electrocoagulation treatment to remove heavy metals (zinc, copper, and chromium) from wastewater generated during the washing process of the screen printing plate was carried out. The electrocoagulation efficiency is estimated based on reducing the concentrations of detected heavy metals in the screen wastewater at defined process parameters such as electrode material, current density, interelectrode distance, and operating times.

Introduction

After the screen printing process is completed, it is necessary to wash the screen plate to remove the leftovers of the used printing inks. For that purpose, special chemicals are used as a combination of different solvents, thinners, and alkaline detergents. Special chemicals are applied to the plate with a sponge or brush, and after a certain period, they are rinsed with water [1]. In this way, a screen effluent is generated.

Experimental

The electrocoagulation cell is made of borosilicate glass with a volume of 250 mL. Two plate iron and two plate aluminum electrodes with the exact dimensions of 10 cm x 5 cm x 0.1 cm and a total area of 100 cm² were used. In Fe(-)/Al/Fe/Al(+) electrode combination, only the outer electrodes were connected in a bipolar mode to a digital DC power supply (DF 1730LCD) equipped with potentiostatic or galvanostatic operational options. 220 mL of the screen wastewater were mixed with the appropriate amount of sodium chloride (0.50 g), at 450 rpm by a magnetic stirrer (IKA color squid, Germany). The current density of 8 mA cm⁻² and the interelectrode distance of 1.0 cm were applied. Electrolyte samples (15 mL) were taken at certain treatment times (1, 5, 10, 20, 40, and 60 min). All collected samples were centrifuged for 10 min at 2000 rpm (Centrifuge Tehtnica Železniki, Slovenia). The Atomic Absorption Spectroscopy (PerkinElmer Analyst 700, USA), according to the standard EPA 7000B method, was carried out for heavy metal analyses [2,3].

The efficiency of electrocoagulation removal of heavy metals (zinc, copper, and chromium) from the screen wastewater was determined by using the equation [2]:

$$E_M = \frac{C_o - C_t}{C_o} \cdot 100 \quad (\%)$$

Where:

 $E_M(\%)$ – the electrocoagulation removal efficiency of a suitable heavy metal,

 $C_o (\text{mg L}^{-1})$ and $C_t (\text{mg L}^{-1})$ – concentrations of analyzed heavy metal in the screen wastewater before electrocoagulation and at a particular operation time (*t*), respectively.

Results and discussion

Efficiencies of electrocoagulation treatment removal of analyzed heavy metals from screen wastewater at precisely defined operating parameters (system with Fe and Al electrodes, current density of 8 mA cm⁻², interelectrode distance of 1 cm, and process times of 1, 5, 10, 20, 40, and 60 minutes) are shown in Figure 1.



Figure 1. Efficiencies of electrocoagulation removal of zinc, copper, and chromium from screen wastewater

The obtained results of electrocoagulation treatment show that:

- The efficiencies of heavy metals removal decrease in order: copper > zinc > chromium. Thus, the nature of the metal affects the order of removal.
- The efficiency of all heavy metals not linear increases with increasing operating time.
- The operating process times of all three heavy metals are 60 minutes.

Conclusion

It is possible to remove heavy metals (zinc, copper, and chromium) from the wastewater generated while washing the screen printing plate by applying electrocoagulation treatment. Maximum efficiencies of electrocoagulation treatment with defined operating parameters for copper, zinc, and chromium were 87.3, 75.3, and 50.0%, respectively.

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CERAMICS-BASED CATALYST CHARACTERIZATION Owais al-aqtash, András Sápi

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Abstract

As the air pollution generated from mobile sources is a problem of a general interest, Toadies' restrictions on pollutant emissions require the use of catalytic convertor as a standard on car engines both SI and CI. Due to incomplete combustion in the engines, there are a number of products CO, HC, NOx, etc. that have a negative impact on air quality. The scope of this paper is to characterize the performance of catalysts with different basis (aluminum oxide, ceramics and zeolite) and different shapes while infused with a fixed percentage of Palladium at 0.2% on the reduction of these harmful gases. To this purpose a two cylindrical Benzene engine was utilized and a gas sensor were attached to the outlet of the exhaust system before and after the catalytic convertor. The model has also given access to the amount of pressure drop caused by the catalyst parts which are compared in the paper.

TRACE ELEMENT DISTRIBUTION MAPPING IN PHARMACEUTICAL AND BIOLOGICAL SAMPLES USING LASER-INDUCED BREAKDOWN SPECTROSCOPY

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Introduction

Laser-induced breakdown spectroscopy (LIBS) has numerous advantages like determination of ppb-ppm level concentrations, microdestructivity, the ability of making high resolution laterally (μ m) or depth-resolved (100 nm) analysis, sensitive measurement of heavy and light elements and direct analysis of solid samples with minimal sample preparation. The aim of the present work was to demonstrate that LIBS is a suitable analytical technique to assess the trace element distribution of pharmaceutical and biological samples. As test samples, we used specimens of chickpea milkvetch (*Astragalus cicer*), a perennial plant with excellent nutritional value and a commercially available dietary supplement pill (Supradyn).

Experimental

The LIBS spectra were taken by using the J200 LA-LIBS tandem spectrometer (Applied Spectra Inc., USA) with the following settings: laser pulse energy 14 mJ, spot size 60 μ m, integration time 1.05 ms, gate delay 0.5 μ s, repetition rate 10 Hz. The laser parameters were set to collect data from the whole sample surface with no overlapping measurement points, and from each location, only one spectrum were recorded.

The only sample preparation in the case of pill was cutting it in half with a sharp scalpel and embedding it in bluetech glue in order to fix them in the sample holder. Seeds of *Astragalus cicer* were surface sterilized with 20% (v/v) sodium hypochlorite for 20 min, and washed with sterile distilled water four times in 20 min. Seeds were dried on a sterile metal filter and we polished them one by one using P-400 sanding paper in order to scratch the external seed coat. Seeds were placed on agar medium (the scratched surface of the seeds contacted the medium). Plastic, square Petri dishes contained half-strength Murashige and Skoog medium (0.8% (v/v) agar, 1% sucrose). Samples were grown under controlled conditions (12 h/12 h light/dark cycle, relative humidity 55–60% and temperature $25 \pm 2^{\circ}$ C) for 14 days. When the plants grew big enough, the plantlets were carefully removed with tweezers and their roots were rinsed with deionized water to wash away the remaining of the medium in which they were cultivated. Plantlets were then pressed, dried and mounted on a glass microscope slide with double-sided foam tape.

Results and discussion

In Figure 1., the elemental distribution maps of five trace elements (Ca, Mg, Cu, Mo and Zn), are presented in the pill. On the elemental distribution maps, the colour scale ranges from blue to red, where blue indicates low intensity and red indicates high intensity. As it ought to be, the

images testify that all of the elemenst are distributed in a more or less homogenous way troughout the pill. Based on the certificate of the manufacturer, Ca and Mg are present in the pill at the highest concentration (51.3 and 21.2 mg per pill), whereas Cu, Mo and Zn are present at least an order of magnitude lower concentration. This also reflects in the intensities of the elements in the maps, however please note that there are significant differences between the sensitivity of the spectral lines used for plotting as well. Since the pills are made by mixing and pressing together powdered chemicals containing these elements (and others), whether we can detect an element or not also depends on the size of the grains. Interestingly, Cu and Zn appears to be present in a significant amount in the coating of the pill as well, as opposed to the other elements which occur only in the main body of the pill. Ca, Mg and Mo seems to be present in larger grains than the other two elements. These information can help the formulation and quality control of the manufacturing of such pills.



Figure 1. Optical microscopy image (top left corner) and LIBS elemental distribution maps of some of the trace elements in a multivitamin dietary supplement pill.

On the elemental distribution maps, presented in Figure 2., the distribution of four selected essential micro Fe, Mn and macro K, Mg nutritious elements can be seen in *Astragalus cicer* seedlings. The macroelements are presented in the plants in the range of mg/g concentration whereas the

microelements are in the range of $\mu g/g$. It appears that the distribution of these elements is nearly homogenous throughout the whole plant except in the case of Mn. Manganese is mainly enriched in the roots and to some extent also in the lower part of the stem. The cotyledons do not show significant manganese concentration. Mn is presented in a lowest concentration in the plants thus it has the lowest intensities from these four elements. K is an important macroelement involved in osmoregulation that therefore has the highest concentration therefore it is represented with the highest intensity. Similarly, the macroelement Mg being a structural element in chlorophyll pigments and involved in biochemical processes show consistent distribution in the seedlings. Regarding Fe, a similarly equable distribution could be observed reflecting the general necessity of this microelement in plant organs. The distribution of all of the four selected elements shows a common feature: higher concentrations were recorded for the center (inner part) of the root and the cotyledons and they were found to decrease towards the edges. It is clearly visible that these elements are more concentrated in the root than in the cotyledon. Each map has a well-illustrated shape of the root of the plant. With this non invasive and in situ technique we can get extra information from the distribution of the elemental content of the plants with minimal sample preparation compared to classical ICP-MS measurements where complicated sample preparation when digestion of the sample is a must.



Figure 2. Optical microscopy image (top) and LIBS elemental distribution maps of the plant sample

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ENHANCED ELECTROCHEMICAL DETECTION OF ANTI-INFLAMMATORY PHARMACEUTICALS FROM WATER USING ADVANCED VOLTAMMETRIC DETECTION METHODS

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Abstract

Pharmaceuticals and personal care products as pollutants (PPCPs) have been detected in the environment in the last decades. The major concerns with the ecotoxicities of PPCPs come from prescription and over-the-counter medications due to their specific targets on living tissues. In this study, the influence of the operating conditions of the voltammetric techniques, i.e., cyclic voltammetry (CV), differential-pulsed voltammetry (DPV) and square-wave voltammetry (SWV) on the electroanalytical performance of fullerene-carbon nanofiber paste electrode (F-CNF) for two anti-inflammatory, ibuprofen (IBP), and naproxen (NPX) determination is studied. From the cyclic voltammetry characterization, it can be seen that NPX oxidation occurred in two steps, starting with the potential value of +0.9V, followed by the oxidation at the potential value of 1.16V vs Ag/AgCl. The IBP oxidation occurred at more positive potential, at the potential value of +1.25V vs Ag/AgCl, informing about its difficulty to be oxidized. The optimization of the step potential (SP) and the modulation amplitude (MA) were achieved for DPV, which were further applied for SWV technique that exhibited fastest voltammetric response. The best performance in term of the lowest limit of detection (0.5 nM) was achieved for NPX determination using SWV technique at the potential value of +1.05V and, the lowest limit of detection (0.6 nM) was achieved for IBP using optimized DPV technique at potential value of +1.3V. This electrode has a great potential for practical utility in NPX and IBP determination in water at trace concentration levels.

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Cu ,Co, Ni NANOPARTICLES SUPPORTED CERIA CATALYSTS IN AMBIENT PRESSURE CO₂ HYDROGENATION

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Abstract

A series of CeO₂ supported Cu, Co, and Ni catalysts have been synthesized by the wetimpregnation method for CO₂ thermo-catalytic hydrogenation from 200 – 400 °C in the fixed bed reactor. All catalysts were characterized by XRD, N₂-isotherms, and H₂ temperature-programmed reduction. XRD results have suggested that the incorporated Cu, Co, and Ni have uniformly distributed on the CeO₂ surface, N₂-isotherm analysis confirmed that the pores of CeO₂ were blocked by incorporated metals and H₂-TPR indicated strong interaction between active metal and CeO₂. The CO₂ consumption rate and product selectivity depend on the type of active metal on CeO2 and reaction temperature. The order of CO₂ consumption rate for 5wt% catalysts was 5Ni/CeO₂ > 5Co/CeO₂ > 5Cu/CeO₂ at 400 °C. The high CO₂ consumption rate for 5Ni/CeO₂ was attributed to the presence of more number of active metallic Ni during the reaction which dissociated H₂ molecule to H-atoms. The formed H-atoms reacted with active CO₂ molecule and formed CH₄ with 100% selectivity.

Introduction

Carbon dioxide is one of the environmental pollutant gases which is liberated by the use of fossil fuels, high growth of petrochemical and automobile industries. It causes global-warming in the atmosphere. The concentration of CO_2 in the atmosphere can be diminished by the capture and utilization or storage (CCUS) [1]. Among these methods, CO_2 utilization is the most important one. In this method, CO_2 is converted into chemicals and fuels such as CO, hydrocarbons, and 2 alcohols using a solid catalyst [2]. The products are used as fuel and important feedstock in the chemical industry.

 $\begin{array}{l} CO_2 + H_2 \rightarrow CO + H_2O \; \Delta H_{298} \; K = +41 \; kJ/mol \; RWGS \; reaction \\ CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \; \Delta H_{298} \; K = -165 \; kJ/mol \; Sabatier \; reaction \\ CO_2 + H_2 \rightarrow CH_3OH + H_2O \; \Delta H_{298} \; K = -49.5 \; kJ/mol \end{array}$

Methanol synthesis Active metal-supported catalysts such as Pt, Pd, Ru, Rh, Co, and Ni [3-5] have been used for the study of CO₂ catalytic hydrogenation. In these metals, Ru, Rh, Pt, and Pd supported catalyst have shown high CO₂ utilization. However, these metals are very expensive. Therefore, non-noble metals such as Cu, Co, and Ni supported catalysts are useful for CO₂ hydrogenation. The selectivity of CO or CH₄ depends on the type of catalyst, support, and reaction conditions. The CO₂ catalytic hydrogenation at high-temperature results in coke formation on the surface of the catalyst which deactivates the active metal. It can be overcome by the use of selective support. Metal oxides like Al₂O₃, ZrO₂, SiO₂, carbon materials, CeO₂, TiO₂, and MnO₂ [6-9] were used as supports to deposit the active metals for the study of CO₂ catalytic hydrogenation. Among these supports, CeO₂ has high oxygen storage capacity and

redox property which enhances the catalytic activity [10]. T.A. Le et al have studied CO and CO₂ hydrogenation over Ni supported on different supports such as SiO₂, TiO₂, γ -Al₂O₃, ZrO₂, and CeO₂ [11]. In this article, we have chosen CeO₂ as the support and incorporated different non-noble metals like Cu, Co, and Ni to find out CO₂ consumption rate in CO₂ thermo-catalytic hydrogenation and selectivity of the products CO or CH₄ in the temperature range from 225 – 400 °C in the fixed bed reactor under atmospheric pressure.

Experimental

The CeO₂ supported Cu, Co, and Ni catalysts were synthesized by the incipient wet impregnation method. The Rigaku Miniflex-II X-ray diffractometer was used to record the X-ray diffractions of CeO2 supported catalysts using Ni filtered Cu K α radiation having tube voltage 30 KV and current 15 mA. The Quantachrome NOVA 3000e gas adsorption analyzer was used to measure N₂ adsorption-desorption isotherms at 77 K. The hydrogen temperature-programmed reduction (H₂ -TPR) was carried out using the Quantachrome Autosorb-iQ instrument. The CO₂ thermo-catalytic hydrogenation has been studied in the fixed bed. About, 0.15 g of the catalyst was loaded at the center of the reactor, CO₂ /H₂ (1:4 vol. %) flow rate 50 mL/min, and temperature 200 – 400 °C were maintained. Before studying the reaction, Cu and Ni catalysts were reduced at 400 °C for 2h and Co catalysts were reduced at 500 °C for 2h. The composition of the gas came out from the reactor was analyzed by online-gas chromatography Agilent 6890N having a thermal-conductivity detector and flame-ionization detector.

Results and discussion

Fig. 1 shows the XRD patterns of CeO_2 supported Cu, Co, and Ni catalysts. In Cu, Co and Ni supported on CeO_2 , the diffraction peaks of CuO, Co_3O_4 , and NiO have not appeared which indicated that incorporated metal oxides were highly distributed on the surface of CeO_2 or not in the detection limit of XRD. The textural properties are presented in table 1.



Figure 1. XRD of CeO2 supported Cu, Co, and Ni catalysts

Sample	Surface area	Average pore size	Total pore volume
	(m^{2}/g)	(nm)	(cm^3/g)
CeO ₂	139.5	8.95	0.28
1Cu/CeO ₂	132.9	8.17	0.27
5Cu/CeO ₂	131.6	8.12	0.26
10Cu/CeO ₂	122.1	8.4	0.25
$1Co/CeO_2$	132.4	7.9	0.26
5Co/CeO ₂	130.8	8.0	0.25
$10Co/CeO_2$	121.9	8.1	0.24
1Ni/CeO ₂	133.3	8.0	0.27
5Ni/CeO ₂	129.4	8.1	0.26
10Ni/CeO ₂	124.7	8.3	0.25

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Table 1 Textural properties of bulk CeO₂ and CeO₂ supported Cu, Co, and Ni catalysts

For the comparison study, the CO₂ consumption rates of 5Cu/CeO₂, 5Co/CeO₂, and 5Ni/CeO₂ catalysts at 400 °C were presented in Fig. 2. At most of the temperatures, 5Ni/CeO₂ has obtained a high CO₂ consumption rate compared with other catalysts. The order of CO₂ consumption rate was 5Ni/CeO₂ > 5Co/CeO₂ > 5Cu/CeO₂. The metallic Ni was more active towards dissociation of H₂ molecule to H-atoms which reacted with more active CO₂ molecules. Hence, it showed a high CO₂ consumption rate compared with other metals Cu and Co supported on CeO₂.



Figure 2. CO₂ consumption rate of 5Cu/CeO₂, 5Co/CeO₂, and 5Ni/CeO₂ catalysts

Conclusion

In this work, we have reported the CO₂ consumption rate of CeO₂ supported Cu, Co, and Ni catalysts in CO₂ thermo-catalytic hydrogenation. The characterization results have confirmed the existence of active metals and strong interaction with CeO₂. The Ni supported catalysts have shown a high CO₂ consumption rate compared with Co/CeO₂ and Cu/CeO₂ catalysts. The selectivity of CH₄ was higher for Co and Ni supported on CeO₂ whereas CO selectivity was higher for Cu supported on CeO₂. Hence, the type of active metal and nature of support has influenced the CO₂ consumption rate and selectivity of the product.

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PREPARATION OF PHOTOCATALYSTS BY ATOMIC LAYER DEPOSITION

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Abstract

The use of semiconductor-based photocatalysts (e.g. metal oxides) in sewage water treatment is expected to be of great interest despite its shortcomings [1]. High surface area organic semiconductor-based structures are promising alternatives for the photodegradation of various organic pollutants. In addition, these polymer structures could be great backbones to produce polymer/metal oxide composite photocatalysts by Atomic Layer Deposition (ALD).

Introduction

Organic-based semiconductors, such as conjugated polymers are very appealing alternatives of the traditional metal-oxide-based photocatalysts, with easier recovery and visible light excitability. Forming these polymers in a PolyHIPE (High Internal Phase Emulsion) structure is a great way to produce high surface area photochemically active backbones with great mechanical stability [2].

The atomic layer deposition is a novel thin layer or nanoparticle synthesis method which is based on the reaction of the gas-phased precursors adsorbed on the surface of the substrate. By changing the number of the cycles, the thickness of the layer or the size of the nanoparticle is finely tunable. With this method, it is possible to deposit metals, metal oxides, selenides, sulfides etc [3].

Experimental

The composites were synthesized by atomic layer deposition, depositing titanium dioxide and zinc oxide layers on the conjugated polymer support with different cycle number. The Beneq TFS 200 ALD equipment was used during the process. The as-synthetized composites were characterized by Scanning electron microscopy (SEM), thermogravimetric analysis (TGA) and X-ray diffraction (XRD).

Results and discussion

On figure 1 (a) we can see the thermogravimetric curve of the 300 cycle TiO_2 , where the amount of titanate was 12,13%.



Figure 1. The thermogravimetric curve (a) and the X-ray diffractogram (b) of the 300 cycle ALD synthetized catalyst.

On figure 1. (b) we can see the X-ray diffractogram of the 300 cycle composite catalyst. The reflexions of the (101), (004), (200), (105), (201), (204) Miller index planes of the anatase are clearly visible, which indicates that crystalline anatase formed on the surface of the polymer backbone.

Conclusion

We successfully synthetized TiO₂/polymer and ZnO/polymer composite photocatalysts and found the ideal cycle number to prepare our samples with the desired amount of oxide. The assynthetized catalysts were characterized by SEM, TGA and XRD.

Acknowledgements

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NOISE AND EXTRA ENVIRONMENTAL MEASUREMENTS IN THE CITY OF SZEGED – A 9 YEAR SURVEY

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Abstract

Measurements were carried out on selected points of Szeged to achieve a raw noise distribution of the city. The aim of the measurements was to obtain the environmental noise load values produced by traffic mostly. The first measurements were carried out in 2012 and they were repeated on the same location spots in 2015, 2018 and 2021.

Introduction

Almost all the information someone collects is acquired through sight and hearing (about 83% percent through sight and 11% through the hearing). At the present time more and more people live in large, crowded cities. This artificial environment – compared to the natural environment – can be noisy. It can be even harmful to the hearing. It is a good idea to check from time to time that our environment is still within the healthy limits.

The normal hearing of humans ranges from 20 Hz frequency to 20000 Hz.[1]

The hearing itself is logarithmic. The industrial tools for noise measurements are based on sound pressure level and the data are given in decibel (dB) scale.[2] It is given by Eq.1:

$$L_p = 20 \log_{10} \left(\frac{\Delta p}{p_0}\right) \, dB \tag{1}$$

where Δp is the sound pressure fluctuation, and p_0 is the reference pressure fluctuation value (audition threshold); $p_0 = 20 \mu Pa$.

L(SPL) (dB)	phenomenon
0	audition threshold; mosquito at 3 m
10	human breathing at 3 m
30	theatrical stillness
40	living area at night; stillness of nature
60	office room
70	street traffic at 5 m
90	noise in factory
100	jackhammer at 1 m; disco inside
120	train horn at 10 m
130	pain threshold

Table 1 shows some common examples for easy comparison.

 Table 1. Sound pressure level examples

The 85 dB or higher sound pressure level over a long-term exposure can cause permanent hearing damage. This damage is cumulative throughout the entire life.

The auditory sensation depends on the frequency of the sound strongly: at the same sound pressure level a 200 Hz sound feels much weaker than a 1000 Hz sound. It can be visualized

by the equal loudness curves which are measured first by Harvey Fletcher and Wilden A. Munson in 1933. It was repeated between the year 2000 and 2003.[3][4][5][6]

Professional noise meters use weighting curves to show similar responses to the human hearing. The A-weighting is used for auditory purposes. The C-weighting is almost flat; that can be used to measure the real physical sound pressure values.[7]

Though +6 dB means twice the power, the human perception works in a separate way. If a sound is observed two times louder than the previous one – it means +10 dB higher level.[8][9] From 2018, the measurements were extended to acquire samples of the CO_2 and CO concentrations of the air. So far all CO measurements were 0 ppm.

The atmospheric level of CO_2 is 414 ppm by Mauna Loa Observatory, Hawaii (NOAA-ESRL) [10]; the normal value of CO_2 -concentration at sea level is 250-350 ppm by industrial recommendations. [11]

Experimental

Figure 1 illustrates the different locations in Szeged where the measurements were carried out. Locations 1, 3 and 5 are close to main roads in Szeged with heavy traffic. Locations 2 and 6 are near to less important roads, but sometimes they have heavy traffic, too. Location 4 is chosen to be far away from any traffic; it is among housing blocks (sleeping area – no heavy daytime activity). The measurements contain morning, mid-day and evening data.

Just out of curiosity the noise levels around the area of the Youth Days of Szeged (red area in the map) were measured, too. This festival is held in each August. Locations 7 and 8 are used only for these measurements.



Figure 1. Locations of the measurements in Szeged

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location	description (and GPS coordinates)
1	intersection of roads "Székely sor" and "Temesvári krt." (46° 14.728' N; 020°
	09.842'E)
2	close to the inner city bridge (46° 14.991' N ; 020° 09.605' E)
3	intersection of roads "Római krt." and "József Attila sgt."
	(46° 15.696' N ; 020° 09.479' E)
4	near a housing block, "Csaba u. 43" (46° 15.732' N; 020° 10.116' E)
5	school at "Rókusi krt." near Tesco (46º 16.253' N ; 020º 08.290' E)
6	school at "Boldogasszony sgt. 8" (46° 14.759' N; 020° 09.738' E)
7	parking area at the bridge (46° 14.933' N; 020° 09.410' E) – only for YDS
8	museum garden (46° 15.124' N; 020° 09.162' E) – only for YDS

Table 2. Measurement locations and descriptions.

Results and discussion

The following tables show the current measured data for each location and all the previous noise and CO_2 values.[12]

location 1	Year	workday morning	workday daytime	workday evening
		(7:00-8:00)	(12:00-15:00)	(20:00-22:00)
Sound level (dB)	2012	61.9 ± 5.2	55.5 ± 7.1	52.9 ± 6.8
Sound level (dB)	2015	64.6 ± 6.0	58.0 ± 8.0	52.2 ± 7.7
Sound level (dB)	2018	58.6 ± 7.6	56.1 ± 7.3	50.7 ± 7.0
$CO_2(ppm)$	2018	286 ± 3	189 ± 9	268 ± 6
Sound level (dB)	2021	61.7 ± 6.3	60.0 ± 6.8	56.6 ± 8.1
$CO_2(ppm)$	2021	385 ± 5	255 ± 6	391 ± 10

Table 3. Measurements on location 1.

location 2	Year	workday morning	workday daytime	workday evening
		(7:00-8:00)	(12:00-15:00)	(20:00-22:00)
Sound level (dB)	2012	59.2 ± 7.3	57.4 ± 7.8	54.9 ± 8.1
Sound level (dB)	2015	60.7 ± 8.5	56.9 ± 9.9	55.6 ± 7.1
Sound level (dB)	2019	59.3 ± 7.7	60.1 ± 6.0	50.1 ± 6.4
$CO_2(ppm)$	2018	293 ± 17	193 ± 2	273 ± 20
Sound level (dB)	2021	57.9 ± 5.6	61.6 ± 8.1	54.3 ± 6.2
$CO_2(ppm)$	2021	390 ± 19	301 ± 6	330 ± 6

Table 4. Measurements on location 2.

location 3	Year	workday morning	workday daytime	workday evening
		(7:00-8:00)	(12:00-15:00)	(20:00-22:00)
Sound level (dB)	2012	64.4 ± 3.4	63.4 ± 4.3	60.0 ± 4.9
Sound level (dB)	2015	67.5 ± 2.7	65.1 ± 4.0	60.1 ± 5.0
Sound level (dB)	2019	66.0 ± 3.7	64.9 ± 4.1	61.5 ± 4.7
$CO_2(ppm)$	2018	282 ± 15	174 ± 4	258 ± 5
Sound level (dB)	2021	67.8 ± 2.8	66.9 ± 2.7	65.9 ± 3.7
$CO_2(ppm)$	2021	416 ± 9	263 ± 18	291 ± 3

Table 5. Measurements on location 3.

location 4	Year	workday morning	workday daytime	workday evening
		(7:00-8:00)	(12:00-15:00)	(20:00-22:00)
Sound level (dB)	2012	43.4 ± 2.1	50.0 ± 3.5	42.9 ± 2.9
Sound level (dB)	2015	44.6 ± 2.1	35.3 ± 2.7	42.1 ± 4.1
Sound level (dB)	2019	41.4 ± 2.5	42.5 ± 2.9	41.9 ± 0.9
$CO_2(ppm)$	2018	270 ± 7	169 ± 20	262 ± 8
Sound level (dB)	2021	52.1 ± 1.3	52.0 ± 2.8	50.4 ± 1.1
$CO_2 (ppm)$	2021	429 ± 5	287 ± 10	309 ± 11

Table 6. Measurements on location 4.

location 5	Year	workday morning	workday daytime	workday evening
		(7:00-8:00)	(12:00-15:00)	(20:00-22:00)
Sound level (dB)	2012	57.5 ± 2.9	49.3 ± 2.5	51.4 ± 3.2
Sound level (dB)	2015	52.1 ± 3.3	51.0 ± 3.3	49.0 ± 3.2
Sound level (dB)	2019	51.2 ± 2.9	52.4 ± 3.0	49.9 ± 3.1
$CO_2(ppm)$	2018	285 ± 12	189 ± 12	266 ± 6
Sound level (dB)	2021	62.8 ± 1.8	55.2 ± 2.2	52.3 ± 4.4
$CO_2(ppm)$	2021	428 ± 4	301 ± 10	311 ± 2

Table 7. Measurements on location 5.

location 6	Year	workday morning	workday daytime	workday evening
		(7:00-8:00)	(12:00-15:00)	(20:00-22:00)
Sound level (dB)	2012	64.0 ± 3.4	59.6 ± 6.2	51.9 ± 8.3
Sound level (dB)	2015	62.9 ± 4.7	63.8 ± 4.6	60.2 ± 4.0
Sound level (dB)	2019	57.4 ± 7.6	60.3 ± 6.7	60.1 ± 3.9
$CO_2(ppm)$	2018	279 ± 7	218 ± 14	237 ± 3
Sound level (dB)	2021	64.4 ± 6.0	63.8 ± 5.9	57.1 ± 7.4
$CO_2(ppm)$	2021	349 ± 23	306 ± 11	323 ± 16

 Table 8. Measurements on location 6.

The measurements to check the noise levels of the Youth Days of Szeged are shown in table 9. The samples were taken in the time period of 22:00-24:00.

Youth Days of	Year	location 1	location 2	location 7	location 8
Szeged					
Sound level (dB)	2012	54.2 ± 6.1	59.0 ± 4.1	59.7 ± 2.4	64.9 ± 2.5
Sound level (dB)	2015	53.8 ± 7.2	60.6 ± 4.4	64.2 ± 2.3	61.3 ± 2.3
Sound level (dB)	2019	49.4 ± 4.3	58.8 ± 5.8	62.5 ± 1.6	64.4 ± 2.0
$CO_2(ppm)$	2018	254 ± 2	256 ± 11	247 ± 4	255 ± 3
Sound level (dB)	2021	56.2 ± 6.5	59.5 ± 6.3	65.0 ± 2.7	70.4 ± 2.1
$CO_2(ppm)$	2021	333 ± 10	279 ± 4	276 ± 7	269 ± 4

Table 9. Measurements during Youth Days of Szeged.

Conclusion

The results show that Szeged is a fine city to live in. It has a somewhat quiet acoustical environment. Even during summer festivals.

Amazingly the structure of the city is very good for the air ventilation, too. The measured CO_2 values are quite low, despite the fact that sometimes the measurements were carried out in *1 m* distance only from the motor vehicles (location 2 and 6). In addition to this, there is a clear indication that the CO_2 values are increasing. On the other hand no CO could be measured. This work aims to measure the environmental load produced mainly by the traffic. Currently there is no real tendency in the data. When more and more electric vehicles will appear in the transportation a strong change may be seen.

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SUSTAINABLE REMOVAL OF TOLPERISONE HYDROCHLORIDE FROM AQUEOUS SUSPENSIONS BY CERIUM-ZIRCONIUM-OXIDE NANOPARTICLES

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Abstract

Pure water scarcity is an emerging global issue. Thus, new efficient and sustainable wastewater treatment techniques are required. In heterogeneous photocatalytic degradation, the photogenerated highly reactive radicals mineralize the present toxic compounds such as pharmaceuticals. CeO₂ is a semiconductor metal oxide that belongs to the first generation of photocatalysts [1]. Its utilization in photocatalysis under simulated sunlight irradiation (SSI) can be improved by the application of CeO_2 in nanoforms, when more economical performances under SSI are reached by tailoring their optical bandgap energy. Another way for the enhancement of the photocatalytic activity of CeO₂ under SSI is the preparation of the CeO₂ heterojunction with another metal oxide semiconductor to reduce the electron-hole pair recombination. The CeO₂-ZrO₂ binary system with a high ceria concentration and cubic fluorite structure is considered a promising photocatalyst-candidate and was tested so far in the photodegradation of Congo red under UV and SSI [2]. In this work, cerium-zirconium mixed oxide (0.8CeO₂-0.2ZrO₂) was prepared by a simple, low-cost, environmentally-friendly twostep mechanochemical synthesis. X-ray diffraction (XRD) analysis found the major phase of cubic CeO₂ with an average crystallite size of around 37 nm, while scanning electron microscopy (SEM) images showed agglomerates of 0.8CeO₂-0.2ZrO₂ composed of spherical nanoparticles (NPs) with sizes in the range of 99-256 nm. Raman spectroscopy confirmed the structural features of the synthesized cerium-zirconium-oxide NPs, while the use of diffusereflectance spectroscopy enabled the estimation of the 3.17 eV optical bandgap energy. The photocatalytic efficiency of the newly synthesized NPs was investigated in the removal of tolperisone hydrochloride (TLP). Experiments were conducted in the presence of 0.8CeO₂-0.2ZrO₂ NPs and under the influence of two types of irradiation (UV and SSI). Furthermore, the effect of the initial pH value was also examined. The obtained results indicate a great potential of these NPs in the removal of TLP from an aqueous environment.

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INFLUENCE OF DETECTED NO2 AND O3 ON AIR QUALITY OF THE CITY OF NOVI SAD

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Abstract

Within regular legislation of the Republic of Serbia, air quality monitoring is done on a daily basis, which we compared with the indicative measurement on a location which is in the vicinity of the measuring station "Novi Sad – Liman". Location of the conducted indicative measurement is in the city park called "Liman park". Mean values of the measured pollutant concentrations of O_3 and NO_2 were taken into account (the indicative measurements were done in a period of one hour). Both measuring points are located near the biggest boulevards which have a lot of traffic activity. Indicative measurements were done using the Aeroqual monitor with a GSE (gas sensitive electrochemical) sensor, while the comparative measurements were took by automatic measuring stations put out by the state. Such a comparison of the measured NO_2 and O_3 concentrations in the air of city of Novi Sad was not done before and it can give us insight into a possible harmful impact on the public health of the citizens of Novi Sad as well as the harmful impact on the environment. Results of the measurements pointed out the variations of air pollutant values; for O_3 a 27.78% and for NO_2 92.78% difference between the two locations that are 450 m apart from one another. The measurements were performed at the same time on the same day, temperature and relative humidity were nearly identical.

Key words: Air Quality, Nitrogen-Dioxide (NO₂), Ozone (O₃), City of Novi Sad

Introduction

City of Novi Sad is the administrative center of the South – Backa district and the autonomous province of Vojvodina. The city is located on the bank of the Danube river, occupying 699 ha with two municipalities, Novi Sad and Petrovaradin. According to the list from 2018. Novi Sad has a population of 402861 and population density of 488 citizens per 1 km². Novi Sad is an urban area with a high degree of economic development and with a low share of agricultural activities [1].

Novi Sad has an extremely favorable natural geographical position, which is confirmed by the fact that it lays on the intersection of roadways and waterways. It is positioned on the east tourist route which connects the north, central and west part of Europe with the Adriatic, Aegean and Black sea. Importance of this route, as well as Novi Sad, is emphasized even more by Corridor VII (Danube river) and Corridor X (Central Europe – Black and Aegean Sea), which have a direct effect on the Novi Sad area. The city lays on a main European road E-75, which is the main artery for the south, central and east Europe [2].

Complex chemical processes that occur in the air under influence of primary air pollutants directly change the air quality. In order to have insight into the quality of air of an urban area such as the city of Novi Sad, an analysis of the pollutants has to be done. Comparative analasys was done in the course of one day on two different locations which are distanced only 450 m from one another (by air). An average value of the measured pollutants was calculated in the

period of one hour (13:00) when most of the insolation is present, when the temperature is highest and when the traffic is the heaviest.

The measurement positions (both indicative and the ones used for comparison) are both close to each other but the indicative measurements were done in a "green zone" - in a city park. On the satellite picture below we can see the two measuring points and their distance.



Picture 1. Satellite view of the two measuring points Indicative measurement (mobile, Novi Sad "Liman park") Automatic measuring station (Novi Sad – Liman)

Experimental

To measure our indicative relevant data regarding to the quality of air, relative to the NO₃ and O₃ pollution, the portable device Aeroqual series 500-monitor was used, which can measure and report changes of the levels of pollution in real time. A non-standard and indicative method was used based on an interchangeable gas sensitive electrochemical sensors (GSE). These GSE sensors permit continuous monitoring of a range of gases at low mixing ratios [3]. The measurements from the static measuring station were taken from the measuring station which was installed by the IPA 2012 sustainable project "Establishment of an integrated environmental monitoring system for air and water quality" [4] which was realized with the "Serbian Environmental Protection Agency (SEPA)".

Results and discussion

The urban part of the city of Novi Sad that was used as a representative sample for the analysis of air quality represents one of the most densely populated areas of Novi Sad with a supplemented green park zone called "Liman park", intended for pastime and recreation of citizens. Spending time in parks on fresh and clean air is of a great importance, considering that the modern man has very little free time for departing urban areas. Green park zones and city park zones are usually the only accessible areas for daily contact with nature and clean air. Observing the common anthropological meaning of adults and children going to the park, which contributes to the development and maintenance of the physical and psychological health, maintaining vitality, quality of life, relaxing and having fun all of this represents the special pastime activity of adults and children. Those are sets of activities which directly affect the modern man, upgrading physical and psychological public health [5].

Comparative analysis of the measured concentrations of ozone (O_3) and nitrogen-dioxide (NO_2) that were done with the portable Aeroqual series 500 device and on the automatic measuring station Novi Sad – Liman are shown in the table below (table 1). The measurements were done in the same time (13:00) of the same day (20.10.2021).

Location	Temperature [°C]	Relative humidity [%]	Ozone (O ₃) [µg/m ³]	Nitrogen- dioxide (NO ₂) [µg/m ³]
Indicative measurement (mobile, Novi Sad – "Liman park")	21.58	41	56	46.21
Automatic measuring station (static, Novi Sad - Liman)	21.71	43.27	71.56	23.97

Table 1. Average values of the measured data

If we take a look at the obtained data on the two locations that were measured in the same time, next is concluded: the temperature was nearly identical, as was the relative humidity with a small increase of 2.27% on the static measuring station Novi Sad – Liman but the concentrations of pollutants ozone and nitrogen-dioxide were at a visible discrepancy. Provided that the location "Liman park" is surrounded by two big four lane boulevards with heavy traffic (one of the boulevards is connected to a regional road) and that the other location is enclosed by the residential and commercial buildings.



The detected presence of NO_2 in "Liman park" is 92.78% more then on the automatic measuring station Novi Sad – Liman, while O_3 measurements are 27.78% less in "Liman park". This extremely visible difference in the detected amount of nitrogen-dioxide in the park is a consequence of the proximity of heavy traffic roads (four lane boulevards) that surround the green zone as well as the high presence of public transportation and high number of cars which still use obsolete propulsions based on diesel and other fossil fuel engines. Additionally, "Liman park" is a green zone that isn't adequately protected by a "green barrier" that should decrease the air pollution inside of it (from the satellite view picture it can be seen that "Liman park" has minimal tree canopy protection). Concentration of the detected O_3 which is 27.78% less on the automatic measuring station is unexpected and can be explained by the fact that the indicative measurements were done in a location which is surrounded by tree canopies, so that less amount of sunlight could penetrate into the measuring zone. The fact that the location of the automatic measuring station Novi Sad – Liman is directly exposed to sunlight and UV rays is also a factor which resulted in an increase of the detected bad ground level ozone.

Presence of detected NO₂ and O₃ pollutants has a harmful effect on the respiratory system of the citizens and also has a negative impact on the environment. Fact is that the content of ozone is increased on warm and sunny days (when people usually spend time in parks and outdoors) which is especially harmful for public health. Vulnerable groups (children and older people) that have diagnosed asthma and other respiratory diseases are the ones most exposed to the harmful effect of bad ground level ozone. Besides these groups, outdoor workers and citizens with vitamin C and vitamin E deficiency are also at risk [6]. Breathing air with a high concentration of NO₂ can irritate airways in the human respiratory system. Such exposures over short periods can aggravate respiratory diseases, particularly asthma, leading to respiratory symptoms (such as coughing, wheezing or difficulty breathing), hospital admissions and visits to emergency rooms. Longer exposures to elevated concentrations of NO₂ may contribute to the development of asthma and potentially increase susceptibility to respiratory infections. People with asthma, as well as children and the elderly are generally at greater risk for the health effects of NO₂ [6].

Conclusion

Air quality control in the urban areas of city of Novi Sad covers two different locations which have diametrically different purposes, one is intended for residency while the other is intended for recreation and relaxation. The fact that a different pollutant amount was measured between these two locations (which are 450 m apart by air) leads us to the assumption that the location directly influences the detected amounts of O_3 and NO_2 . To raise the level of air quality, one of the recommended actions would be the greening of urban areas and the transition of public transport to alternative green energy drives to decrease the pollution that is currently being made by obsolete public transportation as well as personal vehicles that consume fossil fuels. Another recommended action would be the construction of transit roads which would relieve the traffic system of the city and decrease heavy traffic and to encourage the use of bicycles and other man powered transportation devices. Putting a permanent air quality measurement station in the green zones would also be of great help with the analysis of polluntant concentration in these zones.

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COMPARISON OF ADVANCED OXIDATION PROCESSES FOR THE REMOVAL OF TRIMETHOPRIM AND 5-FLUOROURACIL

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Abstract

Two pharmaceuticals, trimethoprim (TRIM), an antibiotic, and 5-fluorouracil (5-FU), a widely used cytostatic, were applied as target substances to compare the efficiency of various AOPs, such as UV (254 nm), UV/VUV (254/185 nm) photolysis, ozonation, and O_3/UV process. Opposite that, there is no significant difference between the molar absorbance of target substances; TRIM transformed very slowly, while 5-FU degradation was fast in 254 nm radiated solutions. The low-intensity 185 nm VUV photons highly increased the transformation and mineralization rate of both components. Ozonation was highly effective for eliminating both compounds. However, the mineralization was limited due to the formation products being resistant to ozone. The O_3/UV process primarily increased the mineralization rate, but no significant change in the transformation rates was observed. Comparing the individual methods in terms of energy consumption, the O_3 , and O_3/UV combination was the most effective for both compounds. The effect of biologically treated wastewater as a real matrix significantly reduced the transformation rate in the case of UV/VUV photolysis, while the matrix effect was moderated for O_3/UV and negligible or even positive for ozonation.

Introduction

Antibiotics are used in huge amounts against bacterial diseases. Due to their frequent use, primarily in animal husbandry, their large quantities are emitted into the wastewater and finally reach the surface waters and groundwater. Consequently, they appear in drinking water sources. The traditional biological water treatment process is not adequate for their complete elimination; thus, it is crucial to investigate and develop additive water treatment processes that can effectively remove these hazardous contaminations.

TRIM, which has been used since the 1960s, is mainly effective in treating urinary tract infections and, at the same time, in the treatment of otitis media and bacterial diseases [1]. Numerous studies report the occurrence of TRIM in the environment, which is a consequence of its relatively low biodegradability; its presence in raw wastewater has been reported by several countries [2, 3]. 5-FU is one of the most frequently used chemotherapeutic antimetabolite [4, 5]. In wastewaters from hospitals, it can be detected in 10-100 μ g L⁻¹ concentration.



Fig. 1. Chemical structures of TRIM and 5-FU

In this work, the degradability of these two drug substances with different advanced oxidation processes, such as UV (254 nm) photolysis, UV/VUV (254/185 nm) photolysis, ozonation, and O_3/UV combination, was studied. Low-pressure mercury vapor lamps radiating at 254 nm are widely used for disinfection [6]. The same lamp emitting 185 nm VUV photons besides 254 nm UV is used for TOC decrease and produces high-purity water for electronics and pharmaceutical industries. In water and wastewater treatment, ozonation (O_3) is often used as pre- and post-treatment methods [7] and for water disinfection. The efficiency of ozonation is often enhanced by its combination with 254 nm UV radiation due to the increased radical formation rate.

Experimental

Two low-pressure mercury vapor (LPM) lamps were used as light sources. UV lamp emitting at 254 nm (GCL307T5L/CELL, produced by LightTech, having 227 mm arc length) covered by commercial quartz envelope was used for UV photolysis. For UV/VUV photolysis a lowpressure mercury-vapor lamp having the same electric and geometric parameters (GCL307T5VH/CELL produced by LightTech, having 227 mm arc length) was used. The envelope of this lamp was made of synthetic quartz transmitting 185 nm VUV photons besides 254 nm UV light. The UV (254 nm) photon flux was determined by ferrioxalate actinometry and that was the same ($3.68 \times 10^{-6} \text{ mol}_{photon} \text{ s}^{-1}$) for both LPM lamps. The flux of the 185 nm VUV photons was determined by methanol actinometry and found to be $3.23 \times 10^{-7} \text{ mol}_{photon} \text{ s}^{-1}$. In the case of UV (254 nm), and UV/VUV photolysis, air, oxygen, nitrogen or O₃ containing oxygen was bubbled continuously through the solution. O₃ was produced from O₂ by an Ozomatic Modular 4HC silent electric discharge ozonator. TRIM (Sigma-Aldrich, $\geq 98.5\%$) and 5-FU (Sigma-Aldrich, $\geq 99\%$) solutions with an initial 1.0×10^{-4} mol L⁻¹ solutions were prepared in ultrapure MILLI-Q water (MILLIPORE Milli-Q Direct 8/16), and 500 mL solution was treated in each case.

UV-Vis spectra of the samples were taken with an Agilent 8453 diode array spectrophotometer. Separation of the aromatic components in the treated solutions was performed by Agilent 1100 type HPLC, equipped with a diode array detector (DAD). For the analysis of TRIM and its degradation products, Kinetex 2.6u XB-C18 100A column (Phenomenex) (flow rate 0.75 mL min ⁻¹, the mixture of phosphate buffer and acetonitrile 80-20 v/v%), while for the analysis of 5-FU and its products, Aminex (HPX-87H) column was used to separate 5-FU and its degradation products (flow rate 0.8 mL min ⁻¹, mobile phase: 0.005 M sulfuric acid) was used. The wavelength of the detection was 285 nm for TRIM and 265 nm for 5-FU. Total organic carbon (TOC) measurements were performed using an Analytik Jena N/C 3100 analyzer. The amount of energy required is a model developed by Bolton [8]. In this work, biologically treated wastewater was used to study the matrix effect. Its parameters are collected in Table 1.

рН	7,8
conductivity (μ S cm ⁻³)	1258
COD (mg dm ⁻³)	24,4
$NH_4 - N (mg dm^{-3})$	<0,4
$HCO_3^ C (mg dm^{-3})$	103,4
$NO_3^ N (mg dm^{-3})$	3,37
Cl^{-} (mg dm ⁻³)	120
TOC (mg dm ^{-3})	6,9

Та	ble 1	l:	Parameters	of	biolo	gically	r treat	ed	wastewa	ter

Results and discussion

The efficiency of direct photolysis is determined by the molar absorbance of the target compound at the irradiation wavelength and the value of the quantum yield for its conversion. The difference between the molar absorbance values of the two compounds at 254 nm is negligible: $3650 \text{ M}^{-1} \text{ cm}^{-1}$ for TRIM and $3506 \text{ M}^{-1} \text{ cm}^{-1}$ for 5-FU. However, 5-FU completely converted in 15 minutes (Fig. 2b), less than 20% of TRIM decomposed within 90 min in dissolved O₂ containing solution (Fig. 2a). The low quantum yield of TRIM transformation is most likely due to its intensive fluorescence around 350 nm, in 254 nm irradiated solutions (Fig. 2c). For 5-FU, no fluorescence was observed, which supports that after the absorption of 254 nm photon, a chemical bond cleavage takes place with quantum yield $\Phi = 0.022$. The intensity of fluorescent light decreases by the dissolved O₂ (Fig. 2c), which suggests that the formation of reactive species (${}^{1}O_{2}$, O_{2}^{-} or CO_{3}^{2-}) may be responsible for the slight increase of the transformation rate.



Fig. 2. Kinetics profile of TRIM (a) and 5-FU (b) transformation during UV, UV/VUV, ozonation, and UV/O₃ treatments, absorption spectra, and the effect of O_2 on the emission spectra of TRIM (excitation: 254 nm) (c)

biologically treated domestic wastewater as matrix on the transformation of TRIM and 5-FU									
	ro ^{ref} (×10-	⁻⁷ M s ⁻¹)	E _{EO} (kWh	m ⁻³ order ⁻¹)	Matrix effect (r ₀ /r ₀ ^{ref})				
	TRIM	5-FU	TRIM	5-FU	TRIM	5-FU			
UV	~0.04	2.00		3.5					
UV/VUV	0.83	3.35	10.6	2.1	0.36	0.42			
O 3	4.88	5.75	2.8	0.88	1.47	1.34			
O ₃ /UV	5.28	7.60	2.8	1.4	1.26	0.88			

Table 2: Initial transformation rates (r_0), electrical energy per order (E_{EO}), and effect of biologically treated domestic wastewater as matrix on the transformation of TRIM and 5-FU

 r_0^{ref} : initial transformation rate determined in Milli-Q water; r_0 : initial transformation rate determined in biologically treated domestic wastewater as matrix

During UV photolysis, the dissolved organic compound absorbs the photons, but the 185 nm VUV photons are adsorbed by water and result in the formation of H• and •OH ($\Phi^{185nm} = 0.33$). The low-intensity 185 nm VUV radiation highly increased the transformation and mineralization rate of both substances, most probably due to the reactions with formed radicals. The effect is much more pronounced for TRIM than for 5-FU because of the relatively high contribution of the UV photolysis to the transformation. A significant effect of dissolved O₂ and 185 nm photons on the absorbance at 257 nm (Fig. 3a and b) indicates that both factors affect the quality and quantity of the aromatic products and are required for efficient mineralization.



Fig. 3. The absorbance of the treated solutions determined at 257 nm versus time of treatment

Using ozonation, the transformation rate of both components exceeds that determined for UV/VUV photolysis (Fig. 2a,b and Table 2), but ozonation was less effective for mineralization than UV/VUV photolysis (Fig. 4). At the given pH, mainly molecular O_3 , a quite selective oxidizing agent reacts with organic substances. Thus, the TOC content remained constant after a fast drop of TOC value; finally, no more than 15% TOC can be removed. In contrast, during UV/VUV photolysis, the decrease in TOC is continuous despite the slower conversion rate. The combination of ozonation and UV photolysis generally enhances efficiency due to non-selective and highly reactive •OH formation via UV photolysis of O_3 . The O_3/UV combination did not increase the transformation rate but highly enhanced the mineralization with the formation and transformation of intermediates (Fig. 3).

Regarding energy efficiency (E_{EO}), UV/VUV photolysis, ozonation, and O₃/UV combination proved effective, partly depending on the target compound. In these cases, we also examined the matrix effect using biologically treated domestic wastewater. For UV/VUV photolysis, the matrix decreased the transformation rate, while for ozonation increased that.

The most plausible explanation is that for UV/VUV photolysis, the dominant reaction partner is the non-selective \bullet OH, thus the competition between the matrix components and target substances decreases the transformation rate. While for ozonation matrix components can behave as promotors and enhance the transformation of O₃ and consequently the formation of

reactive oxygen-containing species and transformation of TRIM and 5-FU. In the case of O_3/UV a slightly negative effect for 5-FU degradation was observed since, in this case, both O_3 and radical-based transformation are significant.



Fig. 4. TOC content versus time of treatment

Conclusion

In the present work, two pharmaceuticals, trimethoprim (TRIM), an antibiotic, and 5-fluorouracil (5-FU), a widely used cytostatic, were applied as target substances to compare the efficiency of various AOPs, such as UV (254 nm), UV/VUV (254/185 nm) photolysis, ozonation, and O_3/UV process. The low-intensity VUV light highly enhanced the transformation and mineralization rates. Ozonation was the most efficient for transforming target substances, but it was pretty inefficient for mineralization. In terms of transformation, mineralization, and electrical energy efficiency, the O_3/UV treatment was the most efficient. Moreover, biologically treated wastewater as a matrix just slightly affected the efficiency of this method, opposite to the UV/VUV photolysis.

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AMINO FUNCTIONALIZED GRAPHENE QUANTUM DOTS - NEW FLUORESCENT SENSOR FOR Co²⁺ IONS

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Abstract

Graphene quantum dots (GQDs) were fabricated by simple electrochemical oxidation of graphite electrodes. Modified GQDs were obtained by gamma irradiation of GQDs, EDA, and IPA mixture at a dose of 25 kGy. In this approach, GQDs with the amino groups were produced (25γ -GQDs). These GQDs have shown a high uniformity, good dispersibility in water, and strong photoluminescence in the blue part of the electromagnetic spectrum. Modified GQDs were studied in the optical detection of Co²⁺ ions. The PL intensities of GQDs were measured in the presence of Co²⁺ ions in the concentration range 0-7.5 µmol L⁻¹. It was demonstrated that PL intensities decreased linearly in the concentration range 0-2.5 µmol L⁻¹. The value of the coefficient of determination (R²= 0.95949) indicates the potential of 25γ -GQDs for application in optical sensing of Co²⁺ ions.

Introduction

Due to intensive human activities such as agriculture and industry, a large amount of hazardous substances is present in the environment. Usual pollutants are heavy metals. Cobalt is one of the most widespread trace elements in nature. The only known biological role of cobalt in organisms is that it forms an integral part of vitamin B12 (cyanocobalamin). All other Co compounds are toxic for living beings [1]. Mostly used analytical methods for Co²⁺ detection such as inductively coupled plasma optical emission spectrometry (ICP-OES), colorimetric, and electrochemical methods possess some drawbacks: high-cost instruments, complicated sample preparation, and long duration of the analysis. Therefore, some alternative methods have been developed [2, 3].

Nowadays, photoluminescence (PL) methods attract attention for Co^{2+} ions detection. One of the most investigated materials on the PL detection field are graphene quantum dots (GQDs) [4]. GQDs are 0D carbon nanomaterials built from sp² hybridized C atoms organized in the hexagonal crystal lattice with oxygen contained functional groups (hydroxyl, carboxyl, carbonyl, and epoxy) attached on their surface and edges. The size of GQDs is lower than 10 nm, while the average height is around 2 nm [5]. Thanks to their great features such as absorption of UV and visible light, PL in the visible part of the spectrum, solubility in water and polar solvents, nontoxicity, and biocompatibility, GQDs are widely examined for applications in electronics, bioimaging, drug delivery, and optical sensing [6-8]. The origin of PL in GQDs is still insufficiently explained but is considered that it can be formed by the combination of some different factors: quantum confinement effect, edge effect, functional groups on their surface, etc. [5]. Due to PL, GQDs were investigated as an optical sensor for Co²⁺ ion detection. Boonta et al. produced S, N-doped GQDs for PL detection of bivalent cobalt ions [2]. PL intensities of GQDs were measured in presence of Co^{2+} in the concentration range of 0-60 μ mol L⁻¹. It was observed that PL of GQDs was quenched by Co²⁺ and that intensities of PL decreased linearly from 0 to 40 μ mol L⁻¹.

Herein, we produced pristine graphene quantum dots (p-GQDs) from graphite electrodes in a simple *top-down* electrochemical approach. Then, we successfully introduced amino groups in the structure of GQDs by gamma irradiation at a dose of 25kGy using a mixture of water, ethylenediamine (EDA), and isopropyl (IPA) alcohol as a medium for radiation. In this case, EDA was used as a source of amino groups, while IPA served as a quencher of oxygen radicals during irradiation. Before irradiation, a mixture was bubbled with argon to remove dissolved oxygen. In these conditions, we performed a structural modification of GQDs. Obtained GQDs with amino groups were labeled as 25γ -GQDs. These GQDs were investigated as PL sensors for Co²⁺ ions.

Experimental

Pristine graphene quantum dots (p-GQDs) were synthesized using the previously described electrochemical method [8]. A water dispersion of p-GQDs in the concentration of 1 mg mL⁻¹ was mixed with 4% EDA and 3% IPA. In the final stage, Ar was passed through dispersion. Then, the mixture was exposed to gamma irradiation at a dose of 25 kGy. As a source of gamma irradiation, a Co-60 was used.

To investigate a chemical composition of 25γ -GQDs infrared spectroscopy with Fourier transformation (FTIR) was used. For FTIR analysis, the 25γ -GQDs powder was used. FTIR spectrum was recorded in attenuated total reflection mode (ATR) on the *Thermo Scientific Nicolet 6700 FTIR* instrument (*Thermo Fisher Scientific*, Waltham, MA, United States). The spectral resolution was 2 cm⁻¹.

Morphology of 25γ -GQDs was examined by atomic force microscopy (AFM). Water dispersion of 25γ -GQDs in a concentration of 0.25 mg mL⁻¹ was used for analysis. AFM images were obtained using *Quesant* (Agoura Hills, CA, United States) microscope operating in the tapping mode in air and at room temperature. QWM300 AFM probe, rotated, monolithic, silicon probe for non-contact high-frequency application was used. Standard silicon tips (*Nano and More, Gmbh*, Wetzlar, Germany) with a force constant of 40 N m⁻¹ were used. Mica was used as a substrate for imaging. AFM images were analyzed using *Gwyddion 2.58* software.

The optical properties of 25γ -GQDs were investigated by UV-Vis spectroscopy. Dispersion of 25γ -GQDs was prepared in a concentration of 0.25 mg mL⁻¹. The absorption spectrum was recorded on Shimadzu UV-2600, UV-Visible Spectrophotometer (Shimadzu, Corporation, Tokyo, Japan) at 20° C in the wavelength range 200-800 nm.

For PL measurement, a dispersion of 25γ -GQDs in the concentration of 0.125 mg mL⁻¹ in methanol was prepared. PL spectra of 25γ -GQDs were recorded on *Horiba Jobin Yvon Fluoromax-4* spectrometer (*Horiba*, Kyoto, Japan). PL intensities were measured under the different wavelengths of exciting light in the range 300-400 nm, in air condition, at a temperature of 25°C.

For detection of Co^{2+} ions, water dispersion of 25γ -GQDs in a concentration of 0.03 mg mL⁻¹ was mixed with selected ions in the concentration range of 0-7.5 µmol L⁻¹. The PL intensities of 25γ -GQD in presence of Co^{2+} were measured on *Horiba Jobin Yvon Fluoromax-4* spectrometer (*Horiba*, Kyoto, Japan), under an excitation wavelength of 360 nm. PL spectra were collected at room temperature in air conditions.

Results and discussion

On the UV-Vis spectrum of 25γ -GQDs (Figure 1a) absorption bands at 206 and one shoulder band around 250 nm can be observed. The band at 206 nm is attributed to the π - π * transition of C in aromatic bonds. Shoulder band localized around 250 nm steams from n- π * transitions in C=O bonds. Results obtained by FTIR analysis are presented in Figure 2b. Three lower bands at 2872, 2926, and 2868 cm⁻¹ steam from stretching vibrations of C-H bonds in -CH and -CH₂
functional groups. The band at 1571 cm⁻¹ originates from π -conjugated domains in 25 γ -GQDs. In the FTIR spectrum of pristine GQDs, the band at 1365 cm⁻¹ steams from symmetric stretching of O-C=O groups [8]. In the sample 25 γ -GQDs, this band is shifted at 1373 cm⁻¹ due to reduction of the carboxyl group, under the gamma irradiation. Two bands at 1068 and 3363 cm⁻¹, which steam from stretching vibrations of C-N and N-H bonds, confirmed the incorporation of amino groups in the GQDs structure.



Figure 1. UV-Vis absorption (a) and FTIR (b) spectra of 25γ -GQDs.

AFM images of 25γ -GQDs (Figure 2 a, b, and c) show that these GQDs are spherical, welldispersed nanoparticles. According to their height profiles (Figure 2 d and e), the measured height was between 0.5 and 2 nm which indicates that these dots are structured from one graphene layer.



Figure 2. AFM images (a, b) with height profiles (d, e) and 3D AFM picture (c) of 25γ-GQDs.

Emission spectra of 25γ -GQDs under excitation wavelength 300, 340, 380, and 400 nm are presented in Figure 3. Obtained results show that the maximum emission peak was shifted under the different wavelengths of exciting light. This behavior in literature was described as excitation-depended PL, and it is a consequence of various factors (quantum size effect, etc.) [5]. Maximum of emission peak of 25γ -GQDs was found at 455 nm under an excitation wavelength of 380 nm which means that 25γ -GQDs emit light in the blue part of the spectrum.



Figure 3. PL spectra of 25γ -GQDs under different excitation wavelengths (300, 340, 360 and 400 nm).

Emission spectra of 25γ -GQDs in presence of Co^{2+} in the concentration range 0-7.5 µmol L⁻¹ show that PL intensities decreased with the increase in the concentration of Co^{2+} ions (Figure 4a). It can be assumed that Co^{2+} leads to quenching of PL intensity which can be described by the Stern-Volmer equation:

$$A_0/A = 1 + K_{sv}[Q]$$

where A_0 presents an integrated area under emission peak in absence of quencher, A is the integrated area under the emission peak in presence of quencher, K_{sv} is Stern-Volmer constant which presents a slope of the curve obtained by linear fitting of dependence A_0/A of quencher concentration data.

By the results obtained from linear fitting of A_0/A dependence of quencher concentration data (Figure 4b, c), it can be observed that PL intensity of 25 γ -GQDs decreases linearly in the concentration range 0-2.5 μ mol L⁻¹. The value of the coefficient of determination R² = 0.95949 makes 25 γ -GQDs a good potential sensor for Co²⁺ ions.



Figure 4. PL spectra of 25γ -GQDs a), A₀/A in the dependence of Co²⁺ concentration b), results obtained by linear fitting of experimental data c).

Conclusion

In this work, we successfully introduced amino groups in GQDs structure by gamma irradiation in an easy, low-cost, eco-friendly procedure without using toxic chemicals. The presence of amino groups in GQDs structure was proved by FTIR spectroscopy. 25γ -GQDs emit blue light and were investigated as a PL sensor for Co²⁺ ions. Obtained results show great potential for the use 25γ -GQDs for optical sensing of Co²⁺ ions, and maybe other similar heavy metals.

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SURFACE ACUSTIC WAVE SENSORS FOR GREENHOUSE GAS EMISSION MONITORING

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Abstract

Surface acoustic wave (SAW) and bulk acoustic wave (BAW) sensors are nowadays widely used in a variety of applications and equipments. Due to their high sensitivity, tunable specificity and small size, SAW sensors can be successfully used for applications such as temperature, mass, pressure, humidity or biological sensors. Taking into consideration the possibility of detection of small quantity of gas, but also the possibility of wireless operating mode of such a sensor, SAW based gas sensors have attracted much interest lately [1, 2, 3]. Thus, an ST-X cut quartz substrate at 262 MHz, can achieve a 0.5 ppm sensitivity for NO₂ detection [4], an Y-X cut LiNbO₃ substrate can achieve a 3.5% sensitivity for CO₂ and N₂ [5]. A typical SAW resonator consists of a piezoelectric substrate, onto which pair of micrometer comb-like metallic electrodes is formed. This pair of micrometer electrodes is called the interdigital transducer (IDT) respectively the reflector, forming together a resonant cavity. Considering the piezoelectric effect, a radio frequency input signal will produce an acoustic wave propagating at the surface of the substrate. In turn, the wave generated will produce an electric charge distribution onto the reflector that can be analyzed in terms of radio frequency output signal. The resonant frequency of the device can be altered by the velocity of the surface acoustic wave traveling between IDT and reflector, but also by the geometry of the interdigital pins. The sensibility of the SAW device related to the velocity of the acoustic wave can be exploited in the construction of SAW sensors, in particular the construction of gas sensors. In this paper, we are studying the photolithography of silver delay lines onto a piezoelectric

substrate, through the negative photo-resistor method. The piezoelectric substrate was prior coated with a thick layer of silver using thermal evaporation with Emitech K975X thermal evaporator. A thin film of UV photosensitive coating was form onto the piezoelectric substrate, using the spin coating technique. The image of the interdigitalised transducers was obtained using a mask printed onto transparent printing paper, with a negative colored image in black and white. The main factors that are influencing the quality of the obtained silver electrodes were presented and discussed, as follows: the affect of the speed at which the spin coating is performed, the affect of the photo-resist dilution, the affect of the UV exposure time and the dilution of the solvent used for the chemical etching of the obtained image.

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INVESTIGATION OF UV/S₂O₈²⁻ AND UV/VUV/S₂O₈²⁻ PROCESSES ON THE DEGRADATION OF TRIMETHOPRIM

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Abstract

Several environmental and public health problems are caused by toxic pharmaceuticals with biological activity released into the environment. Conventional biological water treatment methods are generally not effective enough to completely remove pharmaceuticals, especially antibiotics from waters, so advanced oxidation processes should be used as a complementary method. In this study, the removal efficiency of trimethoprim (TRIM) was investigated using UV (254 nm), UV/VUV (254/185 nm) photolysis, and UV/S₂O₈²⁻ and UV/VUV/S₂O₈²⁻ treatments. Opposite to the relatively high molar absorbance of TRIM at 254 nm, the simple UV photolysis was ineffective for its elimination. Its removal and mineralization were significant in the case of UV/VUV photolysis due to the 'OH formation from water via absorption of 185 nm VUV light. The addition of S₂O₈²⁻ highly increased the transformation and mineralization rate in both cases due to the formation of SO₄^{-•}. At the highest concentration of S₂O₈²⁻, both UV and UV/VUV_{185nm} photolysis removed the total TOC content within 45 minutes.

Introduction

The consequences of the release of pharmaceuticals into the environment have become a global problem in the last decade and caused several environmental and public health problems. According to the published literature, the global use of antimicrobials could reach 200,000 tons per year [1], and a significant part of that enters the wastewater. A considerable amount of pharmaceuticals, primarily antibiotics, are also used as veterinary drugs. The conventional biological wastewater treatment method is not suitable for completely removing these components, so the increasing use of antibiotics significantly increases the risk of developing antibiotic-resistant bacteria, dangerous for animals and humans [1]. In this study, trimethoprim (TRIM), a widely used antibiotic, was used as a model compound, detected several times in wastewaters and grey waters [2]. TRIM has been used for treating various infections (e.g., urinary tract infection) since the '60s and is often applied together with sulfonamides to enhance their effect. TRIM consists of a pyrimidine-2,4-diamine and a 1,2,3-trimethoxybenzene ring connected via a methylene bridge (Fig. 1).



Fig. 1. The chemical structure of TRIM

The Advanced Oxidation Processes are chemical water treatment processes based on the radical generation and suitable for removing small amounts of harmful organic contaminants that cannot be removed by biological water treatment. In this work, the transformation and mineralization of TRIM were investigated using UV, UV/VUV, $UV/S_2O_8^{2-}$ and $UV/VUV/S_2O_8^{2-}$ methods. While 254 nm UV radiation is generally used for water disinfection and adsorbed by the dissolved organic and inorganic substances, 185 nm VUV light having small intensity is adsorbed by water and produces 'OH (2.8 V). Persulfate ($S_2O_8^{2-}$) is a potent oxidizing agent (2.1 V) that can be activated by 254 nm UV light to form highly reactive and more potent oxidizing agents, sulfate radicals (SO_4^{-*}) (2.5–3.1 V) [3]. Consequently, in UV/VUV radiated solutions containing $S_2O_8^{2-}$, both 'OH and SO_4^{-*} can form and cause the degradation.

Experimental

Two low low-pressure mercury vapor (LPM) lamps were used as light sources. UV lamp emitting at 254 nm (GCL307T5L/CELL, produced by LightTech, having 227 mm arc length) covered by commercial quartz envelope was used for UV photolysis. For UV/VUV photolysis low-pressure mercury-vapor lamp having the same electric and geometric parameters (GCL307T5VH/CELL produced by LightTech, having 227 mm arc length) was used. The UV/VUV_{185nm} lamp's envelope was synthetic quartz to transmit the VUV_{185nm} photons. The UV (254 nm) photon flux was determined by ferrioxalate actinometry and that was the same ($3.68 \times 10^{-6} \text{ mol}_{\text{photon} s^{-1}}$) for both LPM lamps. The flux of the 185 nm VUV photons was determined by methanol actinometry and found to be $3.23 \times 10^{-7} \text{ mol}_{\text{photon} s^{-1}}$.

In the case of UV (254 nm), and UV/VUV_{185 nm} photolysis, air was bubbled continuously through the solution. Gas bubbling was started at least 20 min before the measurement. TRIM (Sigma-Aldrich, \geq 98.5%) solution with an initial 1.0×10^{-4} mol L⁻¹ concentration was made in ultrapure MILLI-Q water (MILLIPORE Milli-Q Direct 8/16).

Separation of the aromatic components in the treated solutions was performed by Agilent 1100 type HPLC, equipped with a diode array detector (DAD). For the analysis of TRIM and its degradation products, Kinetex 2.6u XB-C18 100A column (Phenomenex) was used at 30 °C. The eluent contains 20% acetonitrile and 80% phosphate buffer, the flow rate was 0.8 mL min ⁻¹, and 20 μ L sample was injected. The wavelength of the detection was 285 nm. Total organic carbon (TOC) measurements were performed using an Analytik Jena N/C 3100 analyzer. The concentration of H₂O₂ was measured with a cuvette test by Merck, having a 0.015 - 6.00 mg L⁻¹ measuring range.

Results and discussion

During 254 nm UV irradiation, direct photolysis is the main pathway for the transformation of TRIM. The efficiency of UV photolysis primarily depends on the molar absorbance of the target compound. Opposite to the relatively high molar absorbance of TRIM at 254 nm (2942 M^{-1} cm⁻¹ [4]), its transformation is negligible during the first 20 minutes due to the very low quantum yield [5]. After this induction period, a slow degradation can be observed in the air-saturated solution. One plausible explanation could be a slow accumulation of low-reactivity species, such as HO₂[•]/O₂^{•-} or CO₃^{•-}.

Opposite to the low intensity, the presence of 185 nm VUV light highly improved the efficiency (Fig. 2.) due to the formation H• and •OH radicals from water:

 $\begin{array}{l} H_{2}O + hv \ (<\!190 \ nm) \rightarrow H^{\bullet} + {}^{\bullet}OH & \Phi({}^{\bullet}OH)_{185nm} = 0.33 \ [6] \\ H_{2}O + hv \ (<\!200 \ nm) \rightarrow \{e^{-}, H_{2}O^{+}\} + H_{2}O \rightarrow \{e^{-}, H_{2}O^{+}\} + (H_{2}O) \rightarrow e_{aq}^{-} + {}^{\bullet}OH + H_{3}O^{+} \\ \Phi(\ e_{aq}^{-})_{185nm} = 0.05 \ [6] \end{array}$

In the presence of dissolved O_2 , H[•] and e_{aq}^- transforms into the less reactive HO_2^{\bullet} and $O_2^{\bullet-}$, and the main reactive species is the [•]OH, which is a non-selective, strong oxidant.

S ₂ O ₈ ²⁻	UV/air	UV/VUV/air	
c (×10 ⁻⁴ M)	r ₀ (×10 ⁻⁷ M/s)	r ₀ (×10 ⁻⁷ M/s)	ro ^{UV} /ro ^{UV/VUV}
0.0	_	0.817	_
5.0	0.860	1.883	0.457
10.0	1.492	3.383	0.441
20.0	2.983	5.267	0.566

Table 1. Initial transformation rates of TRIM at 1.0×10^{-4} M initialconcentration

The addition of $S_2O_8^{2-}$ highly enhanced the transformation rate in the case of both light sources. Due to the 254 nm UV radiation from $S_2O_8^{2-}$ highly reactive sulfate radicals (SO₄⁻) are formed: $S_2O_8^{2-} + h\nu \rightarrow 2 \text{ SO}_4^{--}$. $\Phi(SO_4^{--})_{254nm} = 1.4 \pm 0.3$ [7,8] The SO₄⁻• has similar reactivity to •OH; however, it is more selective and less reactive towards organic substances. The reaction rate constants of SMT with •OH and SO₄•⁻ were calculated from the competition kinetics method by Luo et al. [4] and found to be $6.02\pm0.13\times10^9 \text{ M}^{-1}\text{s}^{-1}$ and $3.88\pm0.07\times10^9 \text{ M}^{-1}\text{s}^{-1}$, respectively. In the LUV/S O_2^{-2-} process, the relative contribution of the direct photologies to the TPIM

In the UV/S₂O₈²⁻ process, the relative contribution of the direct photolysis to the TRIM transformation is negligible, and SO₄•⁻ based transformation is dominant. Comparing the molar absorbances of TRIM (2942 M⁻¹ cm⁻¹) and S₂O₈²⁻ (20–22 M⁻¹ cm⁻¹ [8]), a higher portion of the photons absorbed by TRIM even at the highest concentration of S₂O₈²⁻. (Table 1, Fig. 2). In the case of UV photolysis, the effect of 3×10^{-3} M SO₄•⁻ was also investigated, which is slightly enhanced the tansformation.



Fig 2. Effect of $Na_2S_2O_8$ dosage on the degradation of TRIM in the case of UV (A) and UV/VUV_{185nm} (B) photolysis

In the UV/VUV/S₂O₈²⁻ method, 'OH and SO₄⁻ based reaction also contributes to the transformation. In both cases, the transformation rate of TRIM increases linearly with the initial $S_2O_8^{2-}$ concentration, but the effect of $S_2O_8^{2-}$ addition is more pronounced in the case of UV/VUV irradiation (Fig. 3). Due to the absorption of 185 nm VUV light by the SO₄⁻, the regeneration of the SO₄⁻ was supposed.



Fig 3. Effect of $Na_2S_2O_8$ dosage on the initial transformation rate of TRIM in the case of UV and UV/VUV_{185nm} photolysis

It is essential to study the mineralization of biologically active compounds. During UV photolysis, the mineralization of the treated solution is neglected; the TOC value just slightly decreased (Fig. 4), while in the case of UV/VUV photolysis, the degree of mineralization is significant; the TOC value decreased by approx. 80% (Fig. 4b). With increasing the initial concentration of $S_2O_8^{2-}$, the mineralization rate highly increased in both cases. At the highest applied concentration of $S_2O_8^{2-}$, the transformation rate of TRIM is higher for UV/VUV photolysis (Table 1.), but the mineralization rate became similar (Fig. 4.).



Fig 4. Effect of $Na_2S_2O_8$ dosage on the mineralization of TRIM in the case of UV (A) UV/VUV_{185nm} (B) photolysis

The characterization of methods includes the energy investment; thus, the electrical energy required for the 90% transformation of TRIM was calculated (Table 2.).

		$c(S_2O_8^{2-}) \times 10^{-4} M$			
		0	5	10	20
TRIM ^{90%}	UV	108	27	14	8
	UV/VUV _{185nm}	24	12	8	4
TOC ^{90%}	UV	—	233	142	68
	UV/VUV185 _{nm}	127	108	108	54

Table 2. The electrical energy required for transformation of 90% TRIM (kJ) and 90% of the TOC content at 1.0×10^{-4} M initial concentration

As Table 2. shows, in the case of UV photolysis, even at the lowest concentration of $S_2O_8^{2-}$, the electrical energy consumption was reduced significantly and approached that required for UV/VUV photolysis. Although the transformation rates (Fig. 2.) and the mineralization (Fig. 4.) were similar at the highest $S_2O_8^{2-}$ concentration using UV and UV/VUV light sources, the energy consumption is much lower.

Conclusion

The photolysis of TRIM without the addition of $S_2O_8^{2-}$ was only effective during UV/VUV photolysis. The addition of $S_2O_8^{2-}$ highly increased the transformation and mineralization rates in both cases. At the highest $S_2O_8^{2-}$ (2.0×10⁻³ M) concentration, both UV and UV/VUV photolysis eliminated the TOC within 45 minutes. The electrical energy consumption was lower at each $S_2O_8^{2-}$ concentration using UV/VUV photolysis than UV photolysis, especially for mineralization.

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EFFECT OF INORGANIC IONS ON THE VACUUM-UV PHOTOLYSIS OF WATER

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Abstract

Vacuum-ultraviolet (VUV) photolysis is based on high-energy radiation ($\lambda < 200$ nm) where photons are absorbed by water and produce highly reactive species; primarily HO[•] and H[•], and in smaller quantities e_{aq}^- . VUV photolysis, due to the radicals formed, can efficiently transform and mineralize organic contaminants without any other additives. In this work, the effect of different inorganic ions (Cl⁻, NO₃⁻,HCO₃⁻), present in large amounts in wastewater, was investigated in the case of two different types of VUV light sources. The conventionally used low-pressure mercury-vapor lamp emits both 254 nm UV and 185 nm VUV photons and is widely used in water treatment for disinfection (254 nm) and producing high purity water (254/185 nm). The other applied light source was the Xe^{*} excimer lamp, used mainly in the laboratory scale, emits quasi-monochromatic 172 nm VUV light. The effect of inorganic ions during VUV photolysis depends on the radical scavenging capacity, molar absorbance of ions, and the properties of the radicals and radical ions formed from them by VUV or UV photolysis (for UV/VUV_{185nm}), which is well reflected by the results presented.

Introduction

VUV photolysis is based solely on high-energy ($\lambda < 200 \text{ nm}$) radiation. In the case of VUV photolysis the decomposition and mineralization of organic contaminants are initiated by the reactions with reactive hydroxyl ('OH) and hydrogen radicals ('H) formed during the VUV photolysis of water:

$$\begin{array}{l} H_{2}O + h\nu \ (<\!190 nm) \rightarrow H^{\bullet} + {}^{\bullet}OH \\ H_{2}O + h\nu \ (<\!200 nm) \rightarrow [e^{-}, H_{2}O^{+}] + H_{2}O \rightarrow [e^{-}, H_{2}O^{+}] + (H_{2}O) \rightarrow e_{aq}^{-} + {}^{\bullet}OH + H_{3}O^{+} \ (2) \\ \Phi_{172 \ nm} = \Phi_{185 \ nm} = 0.05 \ [2] \end{array}$$

The quantum yield of the 'OH formation from water is slightly different at 172 nm (0.42) and 185 nm (0.33). A much more significant difference was reported between the absorption coefficients at these wavelengths. The absorption coefficient of water at 185 nm is 1.62 cm^{-1} [3,4], while, at 172 nm, this value is 550 cm⁻¹. Consequently, the penetration depth of VUV radiation is about 11 millimeters for 185 nm, but no more than 0.04 mm for 172 nm [5,6].

It is generally accepted that water absorbed VUV photons exclusively due to its much higher concentration than dissolved substances. Most studies about the application of VUV photolysis to eliminate organic substances from aqueous solutions focus on reactions with primary radicals, mainly 'OH, and do not address the effect of matrix components; experiments are performed mainly in pure aqueous solutions. However, from the practical application point of view, the effect of matrix components must be considered. Moreover, according to recent literature [7], some inorganic ions have significant absorption in the VUV wavelength range, and their molar absorbance highly exceeds that of water. Thus, competition can occur between the inorganic ions and water for the high-energy photons. By acting as a radical scavenger, inorganic ions may also reduce the efficiency of VUV photolysis. Some data about the molar absorbance of inorganic ions at 185 nm were reported, but no data about the molar absorbance of inorganic ions at 172 nm.

This study aims to investigate the effect of various inorganic ions (NO_3^-, Cl^-, HCO_3^-) present in relatively high concentrations in biologically treated wastewater. This work used two types of VUV light sources: the conventionally used low-pressure mercury-vapor (LPM) lamp emitting both 254 nm UV and 185 nm VUV photons, and a Xe^{*} excimer lamp, emitting quasimonochromatic 172 nm VUV light. For LPM lamp, the molar absorbance of inorganic ions not only at 185 nm but also at 254 nm must be considered.

Experimental

The Xe^{*} excimer lamp (130 mm long, 46 mm diameter, 20 W, from Radium XeradexTM) light source for VUV_{172nm} photolysis and LPM (GCL307T5WH, 227 mm arc length, 15 W, from LightTech) for UV/VUV_{185nm} photolysis were used. The VUV photon flux of the lamps was measured by methanol actinometry; the photon flux of 172 nm VUV light was 1.04×10^{-5} mol_{photon} s⁻¹, while the photon flux of 185 nm VUV light was 3.23×10^{-7} mol_{photon} s⁻¹. For the LPM lamp, the photon flux of 254 nm UV light was mol_{photon} s⁻¹, determined by ferrioxalate actinometry. The H₂O₂ concentration was measured with a Spectroquant H₂O₂ cuvette test from Merck, using a Spectroquant Multy spectrophotometer (Merck, SN072188). All high purity salts, used for the investigation were purchased from Sigma-Aldrich, and their purity was≥99.9%.

Results and discussion

During VUV photolysis of water, reactive 'OH and 'H are formed (1,2). In the absence of organic matter and dissolved O_2 ; H_2O_2 can form exclusively by the recombination of 'OH radicals (3)[8]:

 $2 \cdot OH \rightarrow H_2O_2$ $k = 4.0 \times 10^9 - 2.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (3) The dissolved O₂ changes the radical set, due to the addition to H[•], and reaction with e_{ag}⁻:



Fig. 1. The concentration of H_2O_2 as a function of time during UV/VUV_{185nm} and VUV_{172nm} photolysis of pure water

The recombination of HO₂[•] and O₂^{•-} results in H₂O₂ formation:

 $O_2^{\bullet-} + HO_2^{\bullet} + H_2O \rightarrow O_2 + H_2O_2 + HO^ k = 9.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (6) Consequently, dissolved O_2 significantly increases the rate of H_2O_2 formation, partly by inhibiting the recombination of H[•] and [•]OH radicals and mainly by the formation of HO₂[•] and $O_2^{\bullet-}$. The pH is also a key factor because it determines the concentration ratio of $O_2^{\bullet-}$ and $HO_2^{\bullet-}$ (pK_a = 4.8 [9]).

Table 1: Molar absorbance, concentration, relative absorbed photons, the equilibrium
concentration of H ₂ O ₂ , and its initial (r_0) and relative formation rate (r_0/r_0^{ref} , where r_0^{ref} is the
formation rate of H_2O_2 determined in pure water)

				UV/VUV _{185nm}		VUV _{172nm}			
	c ₀ (M)	ϵ_{185nm} (M ⁻¹ cm ⁻¹)	185 nm photons absorbed (%)	r ₀ (×10 ⁻⁸ mol/s)	r0/r0 ^{ref}	Ceq ^{UV/VUV} (×10 ⁻⁵ M)	r ₀ (×10 ⁻⁸ mol/s)	r ₀ /r ₀ re f	c _{eq} ^{VUV} (×10 ⁻⁵ M)
pure water	55.50	1.62	100	5.2	_	0.68	24.7	_	8.54
Cl	2.82×10 ⁻⁴	2791	0.9	4.4	0.85	0.63	23.5	0.95	7.44
Cl	3.40×10 ⁻³		9.5	2.0	0.39	0.49	9.3	0.38	3.73
NO ₃ -	2.42×10 ⁻⁴	5000	1.3	0.93	0.18	0.34	5.9	0.24	5.48
NO ₃ -	1.60×10^{-3}		8.2	0.38	0.07	0.83	3.3	0.13	2.39
HCO ₃ -	3.69×10 ⁻³	269	1.1	3.3	0.64	0.16	8.4	0.34	8.81
HCO ₃ -	8.60×10 ⁻³		2.5	2.6	0.51	0.18	7.7	0.31	8.88

For VUV_{172nm} photolysis, the H₂O₂ concentration changes according to a saturation curve as expected. Both equilibrium concentration and the formation rate determined in O₂-saturated solution exceeded that in O₂-free solution (Fig. 1.). For UV/VUV_{185nm} photolysis, the equilibrium concentration was reached after a maximum value (Fig. 1.), and no H₂O₂ formation was in the O₂-free solution. This kind of time dependence of H₂O₂ concentration is probably due to the additive effect of 254 nm UV light, which causes the photochemical decomposition of the formed $H_2O_2[8]$:

 $H_2O_2 + 254 \text{ nm} \rightarrow 2 \text{ }^{\circ}OH$ $\Phi(^{\circ}OH) = 0.5$ (7)The reaction between H_2O_2 and 'OH consumes 'OH and produce HO_2 ' [8]:

 $H_2O_2 + OH \rightarrow 2 HO_2$

(8)The equilibrium concentration of H₂O₂ is more than ten times higher in the case of excimer lamp due to the much higher photon flux and slightly higher quantum yield of the 'OH formation. However, probably the combination of primary radicals (H' and 'OH) is more pronounced in this case due to the extremely high radical concentration within the thin (0.04 mm) photoreaction zone.

The concentration of Cl⁻, NO₃⁻, and HCO₃⁻ in biologically treated domestic wastewater is around 3.4×10^{-3} M, 2.4×10^{-4} M, and 8.6×10^{-3} M, respectively. In this study, the effect of these anions on H₂O₂ formation was investigated. Two concentration levels were used; one of them was set close to the concentrations of the given ions in the biologically treated water. The other concentration was set to the value where the inorganic ion absorbs between 1% and 10% of the VUV photon.

The Cl⁻ is an effective 'OH scavenger. Its reaction after protonation can result in Cl' [10]:

$$Cl^{-} + OH \rightarrow HOCl^{-} \qquad k = 9.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$
(9)
HOCl^{-} + H^{+} \rightarrow Cl^{+} + H_2O(10)

The direct VUV photolysis of Cl⁻ most probably also result in Cl[•] and $e_{aq}^{-}(7)$ [1]:

$$Cl^- + h \nu_{185 nm} \rightarrow Cl^{\bullet} + e_{aq}^-$$
(11)

In the presence of O_2 the reaction with e_{aq}^- can enhance the H_2O_2 formation via $O_2^{\bullet-}$. Moreover, in the case of LPM lamp, the direct UV photolysis can cause the dissociation of the HOCl, which is the recombination product of Cl[•] and [•]OH. Thus, the effect of Cl⁻ is quite complex and not easily interpreted.

The negative effect of Cl⁻ was observed on the equilibrium concentration of H₂O₂ at its higher concentration only in the case of VUV_{172nm} photolysis (Fig. 1.). However, in the case of UV/VUV_{185nm} , the negative effect is mainly observed at the beginning of the kinetic curve; the equilibrium concentration changes only slightly.



Fig. 2. Effect of Cl^- on the H_2O_2 formation during UV/VUV_{185nm} and VUV_{172nm} photolysis



Fig. 3. Effect of NO_3^- on the H_2O_2 formation during UV/VUV_{185nm} and VUV_{172nm} photolysis

Gonzalez and Braun [11] investigated the VUV photolysis of NO₃⁻ and NO₂⁻. The primary species induced a series of reactions partially depleting NO₂⁻ and NO₃⁻. Transformation rates depended on the presence of O₂, and NO₃⁻, NO₂⁻, peroxynitrite, and N₂O were identified as reaction products after irradiation of NO₂⁻ or NO₃⁻ in aqueous solutions. A reaction mechanism was proposed, where NO₂[•] and NO[•] are key intermediates and include many redox reactions and reaction equilibria. The formed NO_2^- react fast with 'OH [11]:

 $NO_2^- + OH \rightarrow NO_3H$

 $k = 2.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (8)

 $NO_2^- + OH \rightarrow NO_2 + OH$

 $k = 1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (9)

In the case of LPM lamp the UV light must have a significant role besides VUV. The NO₃⁻ absorb 254 nm UV photons (3.51 M^{-1} cm⁻¹), and its UV photolysis (10) increase the amount of 'OH and thus the concentration of $H_2O_2[11]$.

 $NO_3^- + h\nu (254 \text{ nm}) \rightarrow O^{-} + NO_2^{-} + H_2O \rightarrow 2 OH + NO_2^{-}$ (10)In the case of VUV_{172nm} photolysis, the formation rate and the equilibrium concentration of H_2O_2 significantly decreased with NO_3^- concentration. This can be explained by the $NO_2^$ formation and its reaction with 'OH (8,9). The absorption of VUV photons also has a significant contribution to the inhibition of H₂O₂ formation. In the case of UV/VUV_{185nm} photolysis, the lower NO₃⁻ concentration has a negative, while the higher NO₃⁻ concentration has a positive effect on the equilibrium concentration of H_2O_2 , which can be interpreted by the enhanced •OH formation due to direct UV photolysis of $NO_3^{-}(10)$.



Fig. 4. Effect of HCO_3^- on the H_2O_2 formation during UV/VUV_{185nm} and VUV_{172nm} photolysis

 HCO_3^- significantly reduced the initial formation rate of H_2O_2 . HCO_3^- did not affect the equilibrium concentration during VUV_{172nm} photolysis but strongly decreased that in the case of UV/VUV_{185nm} photolysis. The initial concentration of HCO_3^- has no significant effect in both cases. The different behavior can be explained by the significantly different 'OH concentrations and the presence of UV light.

Conclusion

The effect of inorganic ions depends on their radical scavenging capacity, molar absorbance in VUV range, and the properties of the radicals and radical ions formed from them by VUV photolysis and/or UV photolysis (for UV/VUV_{185nm}). This wok represents the significant difference between Cl⁻ NO_3^- and HCO_3^- on the H₂O₂ formation, in the application of high intensity 172 nm VUV and low intensity 185nm VUV radiation. Proper interpretation of the experimental results requires further investigation.

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INFLUENCE OF DIFFERENT PARAMETERS ON DEGRADATION EFFICIENCY OF ANTIBIOTIC DRUG CIPROFLOXACIN

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Abstract

The occurrence of pharmaceuticals in the environment in general, but specifically in the aquatic environment, can have a harmful impact on human health. Hence, it is crucial to prevent their release into the environment [1]. Photocatalysis occurs in the presence of light, i.e. photons of a specific wavelength, and photocatalyst [2]. Compared to other advanced oxidation processes, the process of photocatalytic degradation has proven to be more efficacious in the degradation of pollutants that are otherwise challenging to remove from the environment. Applying photocatalysis, organic pollutants can be degraded and completely mineralized to CO₂, H₂O, and corresponding inorganic ions as products that are less detrimental to the environment in comparison with initial molecules [3]. Ciprofloxacin (Figure 1) is a fluoroquinolone antibiotic commonly used both in human and veterinary medicine for treating various infectious diseases mainly caused by Gram-negative and some Gram-positive bacteria [4].



Figure 1. Structural formula of ciprofloxacin

In this research, we observed the influence of the nature of light on the efficacy of direct photolysis of ciprofloxacin in the commercial formulation (drug Ciprocinal), as well as extent of its photocatalytic degradation using ZnO as a photocatalyst, in the presence and absence of O_2 . Furthermore, the influence of the catalyst loading and the effect of substrate initial concentration on the degradation rate of ciprofloxacin was also examined. The degradation kinetics was monitored by UFLC-DAD.

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USE OF CYCLIC VOLTAMMETRY AS AN EFFECTIVE TOOL FOR MONITORING THE LACCASE CATALYSED POLYMERIZATION OF DIFFERENT LIGNINS IN ACETONE-WATER MIXTURE

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Abstract

The applicability of oxidative laccase for both the degradation and the modification of lignin in aqueous media have been intensively studied but limitations still exists due to the low solubility of lignin in media that are compatible with laccase [1]. Organic water-miscible solvents are often required in laccase-catalyzed oxidations because many of the lignin substrates are insoluble in water. Due to the low electrochemical reduction potential, laccase can only oxidize the phenolic lignin moiety (<20% of total lignin) and not the non-phenolic aromatic structure (80% of total lignin) [2]. Moreover, lignin's microporous network is not readily accessible for laccase molecules due to their large size (~5-6 nm) [3] and further decreases the overall oxidation efficiency. The enzymatic oxidative polymerization of four technical lignins with different molecular properties, Soda Grass/Wheat straw Lignin (SGWL), Organosolv Hardwood Lignin (OHL), Soda Wheat straw Lignin (SWL) and Kraft Softwood (KSL) was studied. All lignins were previously fractionated by acetone/water 50:50 (v/v) and the polymerisation of the low molecular weight fractions (Mw < 4000 g/mol) was carried out in the same solvent system. The comparative electrochemical behavior of laccase-catalysed reactions of different substrate concentrations showed to be effective electron donor in the reduction of dioxygen to water and may help us elucidate the suitable experimental conditions for an effective catalysis of laccase in lignin reactions. The immersion of a laccase-coated glassy carbon electrode (GCE) in the solutions generated large catalytic currents easily recorded by cyclic voltammetry at low potential scan rates. Likewise, it was shown that the current record varied with the substrate concentration and the type of lignin. A polymerization process of lignins on the electrode surface was also observed by the decrease in the oxidation current recorded in the same solution, during a second scan. In order to easily assess the differences in the analytical signals to establish relationships between samples (objects) and points in a signal (variables) the principal component analysis (PCA) was applied to the voltammetric data. The multivariate method PCA was used to simplify the discrimination between the polymerization reactions of lignin samples from different sources to build a method for selection of favourable parameters in the catalytic reactions. Electrochemical studies indicated that the laccase immobilized on glassy carbon electrode surface was able to catalyse the oxidation of lignin in aqueous-acetone (50/50 vol. %) solution.

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AN INTELLIGENT SYSTEM FOR MONITORING IN REAL TIME THE POLLUTANTS FROM RIVERS

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Abstract

In order to identify pollutants in real time, it is required at the level of sampling using automated systems to allow high resolution data monitoring. These are the real state of the quality of a river and allow the detection of both short-term events and long-term changes. At the international level there are specialized systems that monitor water in terms of its pollution. Some of them are made only for pollutants from the agricultural sector, for example the flow of nutrients from rivers [1, 2]; others focused on assessing the ecological status of rivers or the impact of river pollution on drinking water. Most studies have been performed on *in-situ* sensor placement [3, 4]. However, precision water monitoring devices, such as chemical analyzers, are not widely used due to high investment and maintenance costs, as they are used to monitor river basins through analysis stations of national agencies. These types of analyzers are, for example, for determining the amount of phosphorus in water. However, ammonium (NH4-N) and organic carbon (*Total Organic Carbon - TOC*) are of significant relevance to the chemical and ecological status of rivers, as well as to the identification of their sources.



Figure 1: Block schematic diagram of an smart sensor system

The idea of the research starts from the need for real-time monitoring of waters along the entire course and its effluents to assess water quality and determine the sources of pollutants resulting from human activity. Developing of an intelligent system for monitoring in real time the

pollutants parameters of the rivers or urban sewerage systems represents the main aim of this research. In this way, were used modern technologies such as ultra-low power hardware, transmission, processing and storage the data in CLOUD and analysis software solutions of the data based on the artificial intelligence algorithms (Figure 1). Thus, the novelty of the study consist in application and interpretation of an extended set of data, focusing not only on a single type of pollution, but to characterize and classify the entire spectrum of pollution sources using the latest methods and technologies, and possibilities to achieve a competitive product on the market, respectively.

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NANOPARTICLE DEPOSITION BY SPRAY COATING FOR ENHANCED ELEMENTAL MAPPING OF ROCK SAMPLES BY LASER-INDUCED BREAKDOWN SPECTROSCOPY

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Abstract

A novel method development of laser-induced breakdown spectroscopy (LIBS) uses nanoparticles deposition on the sample surface and achieves significant signal improvement (nanoparticle-enhanced LIBS, NELIBS). The present research aims at developing a reproducible method of nanoparticle deposition that would allow spatially resolved NELIBS elemental mapping of solid samples with an improved signal-to-noise ratio. The applicability of the method is demonstrated on a monzogranite rock sample.

Introduction

LIBS is a micro-analytical technique that uses short laser pulses as an energy source to produce a microplasma on the surface of a solid sample, which breaks down sample material and thermally excites the atoms generated to produce an atomic emission spectrum. Nowadays LIBS is being applied in many research fields due to its unique set of characteristics. It is a rapid, sensitive, versatile analytical method, which provides quantitative (ppm level) or qualitative information about the sample, is micro-destructive and requires practically no sample preparation, therefore allows fast and direct analysis of solid and liquid (even gaseous) samples in a non-contact manner. Furthermore, the application of chemical imaging methods or elemental mapping provides a spatial distribution of a single chemical species within a complex sample, obtaining information in the form of 2D (or 3D) images and also offering a high degree of discrimination between different sources [1-3].

Several novel approaches have been proposed for the signal enhancement in LIBS in recent years [3]. Some of them include instrumental arrangements, such as double- or multi-pulse LIBS, microwave assisted- or resonance-enhanced LIBS, but the nanoparticle-enhanced LIBS approach simply uses a deposition of plasmonic nanoparticles (NPs) on metal sample surfaces [4, 5]. The conceptual basis of this approach is that the plasmonic characteristics of the Au, Ag or Cu NPs will largely inrease the electrical field strength of the laser pulse in their close vicinity, which leads to the field emission of electrons from the metallic NPs. These electrons will then facilitate the laser induced breakdown plasma generation as seeds, thereby significantly lowering the breakdown threshold and reserving more of the laser pulse energy for plasma heating, which eventually leads to higher analytical atomic emission signals. Figure 1 illustrates this effect [6].



Figure 1. Sketch of NPs enhanced photoablation in metallic samples [5]

Although NELIBS has been successfully demonstrated as a useful signal enhancement approach for local analysis of solid and liquid samples [7, 8], when simple droplet deposition is applied. NELIBS elemental mapping has not yet been shown to work, due to known problems with the homogeneity of NP distribution with droplet deposition (coffee spot effect). At the same time, LIBS is recently being increasingly explored by geologists and the mining and mineral processing industry. LIBS geochemical analysis is generally directed towards quantitative analysis of the elemental contents of rocks/minerals and identification of the minerals (e.g. mapping of geochemical and mineralogical footprints) [9]. In the present work we set the goal of investigating the applicability of the spray coating nanoparticle deposition method for NELIBS elemental mapping on a monzogranite rock test sample.

Experimental

In the deposition experiments, a 0.05 mg/mL dispersion of Pelco NanoXact 30 nm Au NPs (Ted Pella, USA) was sprayed onto a 10 x 10 mm diced silicon wafer (Ted Pella, USA). The deposition was carried out by a commercial spray gun (DU-30 K) equipped with a 200 μ m nozzle and by employing a 3 bar pressure of argon gas. Surface particle concentration was controlled by the spraying times: 10, 25, 50 and 100 s. The sample was kept at a distance of 25 cm from the nozzle and the spraying was followed by one-minute drying under an infrared lamp. Optical microscopy images of the deposition were taken afterwards with an Olympus BX-43 microscope.

Particle distribution of Au NPs resulting from spray deposition were characterized by scanning electron microscopy (SEM, S-4700, Hitachi, Japan). The quantification of the surface mass concentration of NPs was determined by inductively coupled plasma mass spectrometry (ICP-MS, 7700x, Agilent Technologies, USA). 1 cm x 1 cm Si chips were masked with electrical adhesive tape before the deposition experiments in order to ensure that only the exposed, known surface of the substrate were covered by NPs. After the deposition the chips were subjected to aqua regia (trace quality cc. hydrochloric and cc. nitric acids, VWR Chemicals) treatment, which dissolved the gold NPs. Matrix-matched calibration for the surface area quantification was performed using certified calibration standards (IV-ICPMS-71A and IV-ICPMS-71C, Inorganic Ventures, USA) in the range of 0 ppb – 100 ppb, with the application of 209 Bi as internal standard. All measurements were carried out by monitoring the signal of the 197 Au isotope.

The rock samples were prepared in such a way that from each sample, a 30 mm thin section was cut employing a diamon cutter (Struers DiscoPlan) to form $35 \times 20 \times 10$ mm rectangular bodies for mineral identification by optical microscopy (Olympus BX-43) and the remaining part of the bulk sample was diamond-polished for LIBS analysis. The elemental map was recorded using a J-200 tandem LA/LIBS instrument (Applied Spectra, USA), with a laser spot size of 40 μ m, 266 nm laser wavelength and 17.5 mJ pulse energy. Stepwise scanning was employed and the emission spectrum in the 190 to 1040 nm range was collected. The number

of repeated measurements in one sampling location (without translation) was ten. Measurements were performed at 4–5 sampling points in each mineral grain.

Results and Discussion

Spraying, as a general technical coating procedure, produces a relatively homogenous distribution of droplets on the surface of a solid target (Figure 2, left), consequently we also expected a homogeneous distribution of NPs on the surface after drying, but we carried out a detailed morphological investigation of the depositions. SEM revealed that there is a tendency of NP aggregation (Figure 2, right), which results in an "island-like" deposition pattern. This has already been observed for spray coating in the literature [10], and key factors were also identified that determine the quality of a spray-coated "layer". These are the size and dimensional uniformity of droplets in the spray and the droplet impact velocity. Due to the presence of the liquid phase, there still is a chance that the deposited microdroplets coalesce before they dry, which strongly increases the probability of particle aggregation. It also has to be added that the intuition to strongly lower the NP concentration in the sprayed liquid would result in a longer spraying time in order to achieve the required surface mass concentration, which again would increase the probability of microdroplet coalescence, due to the action of surface tension and gravity.



Figure 2. Optical microscopy (left) and SEM image (right) of a solid sample surface spraycoated with NPs. Please note that the left image shows the droplets deposited before drying, whereas the right image was taken after drying.

Optimization of NELIBS conditions requires a control of the surface mass concentration of deposited nanoparticles [4, 5]. For spray coating the control parameter is the nanodispersion concentration and the spraying time. As it can be seen in Figure 3, the surface concentration range we achieved was from 0.287 to 0.942 μ g/cm². As the time increased, the surface density of NPs increased monotonously, following a linear function for the spray coating deposition. The regression coefficient was 0.7 which indicates a low control of surface concentration, which is probably due to the random coalescence or dripping of the deposited microdroplets. Nevertheless, the optimal mass concentration suggested in the literature [4, 5] could be set on the surface by this process.





Figure 3. Control of the surface concentration of deposited NPs by spray coating, as quantified by ICP-MS analysis.

Figure 4. Reflective optical microscopy image of the rock sample with four minerals indicated by colors.



Figure 5. Intensity-based LIBS elemental maps of a granitoid rock (10 x 15 mm area). Showing Li I 670.7 nm and Mg I 518.3 nm elemental maps of sample without NPs (left side) and with NPs (right side).

Granitoid rocks are of industrial interest, as they contain mineral grains with miningworthy concentrations of Li [9]. The four most common mineral grains in the sample were identified as amphibole, feldspar, biotite and quartz (Figure 4). Subsequently, we tested the optimized spray deposition process (a dispersion of 0.05 mg/mL of Au NPs, spraying in five repetitions each consisting of five seconds of spraying, with a NP surface concentration of $0.347 \ \mu g/cm^2$ on the rock). In order to ensure comparability of the two maps, we first recorded the LIBS map and then applied the spray deposition of gold NPs and performed the NELIBS mapping.

We present two elemental maps for the studied rock sample in Figure 5; the Li map is shown for the spectral line of Li I 670.78 nm, whereas the Mg map is shown for Mg I 518.3 nm. As can be seen, the LIBS mapping of the granitoid rock provides information about the distribution of Li and Mg within the mineral rock; therefore, it is possible to identify the type of mineral that contains most of the targeted trace elements. By the comparison of Figure 4 and 5, it can be observed that biotite and amphibole minerals contain the most Li and Mg. These results suggest that mining for Li in granitoid rocks should be aiming for biotite and amphibole grains. An about two-fold signal enhancement could be achieved by NELIBS. The signal enhancement allowed us to detect minerals grains with smaller concentration of the elements too. For example, quartz, feldspar and biotite grains with low Mg content would also be detected (in the lower left quadrant of the image) when the NELIBS approach was used.

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APPLICATION OF T4 COVALENT FUSION FOR STRUCTURAL STUDIES OF BACTERIAL MEMBRANE TRANSPORTERS

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Investigation of the transmembrane proteins found in bacteria is important, among others, to gain knowledge how to effectively control certain pathogens. Molecular chaperones such as T4 lysozyme or antibodies are found to be efficient for studying the structure of membrane proteins. Our aim is to covalently fuse T4 lysozyme to the C terminus of *Hemophilus influenzae* HI0610 membrane protein to study the structure of this fusion protein by X-ray crystallography after cloning, expression, purification and crystallization.

HI0610 transmembrane protein was cloned into pTTQ18 plasmid by using recombinant DNA technology. T4 lysozyme was covalently fused to the target protein during the cloning. The protein expression was followed by SDS gel-electrophoresis to investigate the presence of the required macromolecule. Optimization of protein expression was also performed where IPTG concentration, temperature and time dependence were tested. Subsequently, the protein was purified by affinity chromatography and the efficiency of Ni-NTA and Talon resins was compared. The target protein was further purified by size exclusion and ion exchange chromatography.

T4 lysozyme was successfully fused to HI0610 fucose-proton symporter by genetic engineering. Gel-electrophoresis confirmed that the target macromolecule was expressed in an appropriate amount. Optimal conditions for protein expression were the following in Luria-Broth medium: 37° C, 12 h of induction time, 0.5 mM IPTG. During affinity chromatography, the target protein was recovered in high purity. Investigating the eluted proteins obtained during protein purification using two affinity columns, we concluded that the Ni-NTA resin bound the target protein more efficiently than that of Co^{2+} ion containing Talon resin. However, after affinity chromatography further purification steps were necessary to reach the highest protein purity. Therefore, size exclusion and cation exchange chromatography were also performed to remove the contaminating proteins.

The final analysis of the purified concentrated sample revealed no detectable contaminations even by the sensitive silver staining and become suitable for crystallization and structural studies.

THE APPLICABILITY OF PHOTOACOUSTIC MEASUREMENT TECHNIQUE FOR QUALITATIVE INVESTIGATION OF DIESEL EMISSION

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Abstract

In our work parallel measurement of wavelength dependent optical absorption, size distribution and number-concentration were produced using multi wavelength photoacoustic spectrometer $(4\lambda$ -PAS) and scanning mobility particle sizer (SMPS). For comparative study two different fuel type was tested at three different working points of the engine. The thermal evolution of the emission was also examined using thermodenuder (TD) unit. Bimodal size distribution of emissions at reference temperature was achieved using pure petroleum-based fuel (B0). The OAC values measured at the operating wavelengths of the instrument decreased with increasing rev and rated torque at all wavelengths. The wavelength dependence quantified by Aerosol Angström Exponent (AAE) can be used here for qualitative analysis of carbon emission and showed increased volatility of organic particles towards higher temperatures.

Introduction

In present days due to their climate and adverse health impact the investigation of diesel particulate matter (DPM) have been in gradually increased scientific interest. The diesel emitted particle is one of the dominant source of light absorbing carbonaceous particulate matter (LAC). which is the second most important climate relevant atmospheric constituent too (Bond et al., 2013). Diesel engines equipped with a modern, sophisticated after-treatment system also meet the new emission standards. However, further restriction of emissions in this way is limited by durability and maintenance. Emission-based fuel development is one of the most promising alternatives not only for reducing emissions but also for more environmentally friendly fuel development. The controlled parameter for soot emitted by a diesel engine is the number and mass concentration that has a limited ability to describe the air quality and climate implications of the DPM assembly. The size distribution, volatile classification and spectral responses of diesel carbon are critical parameters both in climatic and also in health relevancies. A recently introduced in-situ measurement method for volatile classification of DPM is based on measuring the size distribution of a temperature-treated and denuded aerosol assembly (Burtscher et al., 2001). The use of a thermodenuder (TD) for the pretreatment of depleted DPM not only allows the classification of volatiles, but also provides an indirect opportunity to study the state of the particles in relation to a given exhaust gas temperature under steady-state measurement conditions. Measuring aerosol light absorption is also a key, climate-relevant quantity. Moreover, the absorption spectrum of the LAC, which is quantified by its wavelength dependence (AAE) is the only physical quantity that can be measured in real-time and which serves composition and air quality relevant informations (Utry et al., 2014; Ajtai et al., 2015). In this work, we demonstrate the experimental results of number concentration, size distribution and absorption spectra measurement of DPM in the function of the operational condition of diesel engine using different fuel types using scanning mobility particle sizer (SMPS) and multi-wavelength photoacoustic spectrometer (4 λ -PAS). We also study the measured quantities in the function of thermal stability.

Experimental

The experimental set up of sampling system what we used is shown in Fig.1. For the measurement, a four-cylinder EURO 4 PC diesel engine with a 2-liter turbocharged common rail injection system was used to generate diesel exhaust emissions. The rear exhaust gas concentration was further reduced 10-fold with ejector diluter (Palas GmbH VKL 10). In any given operating condition of the engine, the exhaust particles were treated thermally before measurement. In our experiments the measurements were performed with two different fuel compositions (B0 and B7) under three different engine loads (defined on fig. 1 as wp#1, wp#2 and wp#3) at three different denuding temperatures (40°C, 150°C, 300°C). B7 For reference petroleum based B0 (biofree) fuel was used. For the investigation of biofuel effect B0 was blended with 7% FAME (Fatty Acid Methyl Ester) (B7). For reference measurements the heating unit of TD was set to 40°C. While, for volatility measurement the TD was heated to 150°C and 300°C respectively.



Figure 1. The experimental set-up of the sampling system. Operatory conditions of engine, type of fuels, posterior temperature treatment conditions.

Results and discussion

The results of number concentration and size distribution measurements using B0 and B7 fuels at three distinct operational conditions of engine and temperatures are shown in Fig. 2. From the fitted data, we derived the characteristic parameters of the identified modes, including the median count (CMD), the geometric standard deviation (GSD), and the total number concentration (TNC). Total volume concentration (TVC) was also inferred using a simple spherical approach in the calculations. Independently of the fuel used, a bimodal size distribution was identified at a reference temperature (40°C) of 820 rpm at 0 Nm. While at wp#2 and wp#3, a monomodal size distribution achieved at 40°C (Fig. 2). Regardless of engine operating condition and fuel type, the monomodal size distribution has shown similar characteristics than in several previously described diesel soot emission studies (Butcher et al., 2001). In general, regardless of fuel type, engine operating condition, and sample temperature, B7 fuel provides similar emission characteristics (i.e. GMD and GSD values) as B0, but with a total number concentration (TNC) approximately 10-20% lower for all case. In the transition from wp#1 to wp#2, the number concentration decreases significantly while further increasing the torque at a constant engine speed in the transition from wp#2 to wp#3 more or less remained unchanged. It is noteworthy, however, that although the torque increases from 100 Nm to 280 Nm at 2500 rpm (transition from wp#2 to wp#3), the TNC values and the population of particles show a high degree of similarity but varies slightly in population statistics.



Figure 2. Size distribution and its characteristic parameters using B0 and B7 fuels at three different operatory parameters of the test engine. Total Number Concentration (TNC), Count Median Diameter (CMD), Geometric Standard Deviation (GSD). Total Volume Concentration (TVC)

At higher torque (280 Nm), the population contains fewer small (less than GMD) but more large (greater than GMD) particles than at lower torque (100 Nm) at the same speed (2500 rpm) (Fig. 2). This seemingly marginal change as a function of size distribution resulted in significant differences in TVC values. Similarly, despite significant differences in TNC values measured in wp#1 to wp#2 transition the TVC concentration has remained more or less similar. This can also be explained by the differences in the population statistics of the emitted diesel aerosol assembly.

The AAE value deduced from the measured absorption at the operational wavelengths of the multi-wavelength photoacoustic instrument using B0 and B7 fuels at different working points of the engine are drawn in Fig. 3. Based on that the AAE value around 1 means the elemental or black carbon fraction dominancy, while the higher value of that indicates presence of organic or in spectral terminology brown carbon fractions. In this context Fig.3 can be interpreted as the following. The AAE value of B7 is higher than that of B0. This indicates the presence of organic carbon soot with high absorption ability towards the shorter wavelengths. Increasing the rev and torque the AAE value is decreasing and indicates increasing elemental and black carbon dominancy in the emitted aerosol assembly. Finally, the increasing denuder temperature resulted in decreasing AAE values. This tendency means that the volatile organic compounds evaporates from the surface of the particles at higher temperatures.



Figure 3. AAE of diesel emission in the function of working points of engine using different type of fuels and sampling temperature.

Because AAE indirectly determines the ratio of organic to inorganic in the test sample, examining the relationship between the number concentration in the population statistics and the AAE data requires more extensive measurement that goes beyond this work.

Summary and conclusion

The number-concentration and the population statistics including TNC, TVC and GMD values as well as the absorption responses including OAC and AAE values were measured at three different engine operating points using pure petroleum based B0 and B0 blended with 7% FAME content (B7) fuels. The absorption spectra quantified by its wavelength dependency (AAE) were deduced from the measured data at any operating condition of engine and fuel types. We have demonstrated experimentally that the size distribution of the wp#1 has bimodal distribution. We also demonstrated in this work that biofuel content cause higher absorption responses towards the shorter wavelengths and that the TNC values shows increased dynamics than that of AAE values with the increased number of wp's. We also experimentally demonstrated the applicability of the multi-wavelength PA spectroscopy for the emission based fuel development purposes. Finally, we also demonstrated a novel methodology for particle evolution measurement using the combination of PA instrument and a thermodenuder unit.

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EFFECT OF ELECTRON BEAM IRRADIATION AND THE PRESENCE OF ANTIBIOTICS ON THE POPULATION RATIO OF RESISTANT/SENSITIVE BACTERIAL CULTURES IN MODEL WASTEWATER MATRIX WITH ANTIBIOTICS AND BACTERIA ADDED PRIOR TO ADVANCED OXIDATION TREATMENT

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Abstract

Control strategies against the spread of antibiotic resistance should be considered in wastewater treatment plants. It is important to understand how resistant bacteria behave in the presence of trace amounts of antibiotics, in order to implement appropriate measures. In our work, we examined the population dynamics of resistant/sensitive Staphylococcus aureus co-cultures. On the one hand, we gained insight into the effect of trace amounts of antibiotics (piperacillin and erythromycin) on bacteria in different wastewater matrices, and on the other hand, we studied the applicability of electron radiation to eliminate the antibacterial effect. Based on our results, trace amounts of antibiotics act on the resistant strain. Presumably, it triggers biological processes in resistant bacteria that do not provide a competitive benefit but disadvantage over the sensitive subtype, and the trace level of the antibiotic present does not appear to affect the sensitive strains. The effect of these conditions on population dynamics is reduced with the use of accelerated electrons, presumably due to the fact that the decomposition products of the components of the effluent matrix (such as humic acid) also contribute to the chemical transformations. Furthermore, it has become apparent that the presence of trace amounts of antibiotics on the one hand initiates biochemical processes in the resistant subtype and on the other hand sensitizes bacteria to the attack of free radicals generated during electron beam treatment. It is clear that more understanding is needed on the effects of trace level of antibiotics in environmental waters on the cellular response and population behavior of resistant bacterial cultures.

Introduction

Radiation sterilization has been developed as a safe and reliable technology and is widely used in the healthcare sector. By using it, the microbiological safety of food is improved and their shelf life is extended [1-2]. In addition, ionizing radiation has been admitted to be effective for reducing the bacterial load of wastewater and sewage sludge [3-6]. However, it should be contemplated that the species present in these diverse microbial populations show different sensitivities to irradiation [5]. Extensive DNA damage occurs in bacteria as a result of exposure to lethal radiation. These bacteria are unable to multiply. Some recent studies indicate that radiation-inactivated bacteria are still metabolically active. The mechanisms are different when heat sterilization is used [7-9]. Based on these, we assume that sensitive and resistant subtypes of the same bacteria may show different sensitivity to ionizing radiation. Therefore, we further investigated this issue and performed experiments with bacteria present during irradiation.

Experimental

Experiments (Fig. 1) were done in the presence of bacteria added to the wastewater matrix prior to the advanced oxidation treatment. In this case the original synthetic wastewater matrix was prepared with or without the antibiotic (non-concentrated samples).

The electron beam (EB) treatment was performed using a Tesla Linac LPR-4 type linear electron accelerator. Our method is based on a microbiological assay we developed previously [10-11].

We selected sensitive and resistant *Staphylococcus aureus* (*S. aureus*) isolates (National Collection of Agricultural and Industrial Microorganisms, NCAIM, Szent István University) to monitor the change in antibacterial activity. In this test the dynamics of a mixed (sensitive/resistant) bacterial population gives information on the effects of antibiotics in a concentration well below the minimum inhibitory concentration (MIC). Inocula were prepared from an overnight culture (incubated at 37 °C) in the case of the sensitive strain, and in the case of the resistant one, the freshly innoculated cells were incubated for 72 h (at 37 °C) for the bacterial suspension preparation. This incubation time was sufficient to yield a culture containing dead cells with released genetic information, which is then available for the sensitive cells that may acquire resistance. The sensitive and resistant subtypes in a 1:1 ratio were added to the wastewater matrix prior to the advanced oxidation treatment. After the irradiation, the samples were incubated for 24 hours (at 30 °C). Colony counting was performed on tryptocasein soy broth (CASO) agar plates. After spreading 100 μ L samples evenly on the surface, the inoculated plates were incubated at 37 °C for 24 h and then the number of surviving colonies were counted.

The total colony count (sensitive + resistant) was determined on unspiked agar plates, while resistant cells were counted on agar plates spiked with the corresponding antibiotic reaching a concentration well above the minimum inhibitory level.

Only resistant cells grow on the surface of the agar plates containing the antibiotic above the MIC. Then the ratio of resistant colonies to the sensitive + resistant colonies was calculated.

Results and discussion

Using advanced oxidation treatment (in the form of EB irradiation), the selective pressure on the bacterial population favouring the predominance of antibiotic resistant mutants can be eliminated. This is achieved when the fraction of resistant bacteria, within a statistically insignificant deviation, is the same as in the control sample (with no antibiotic added). In other words, the difference between the control sample and the sample containing the antibiotic (piperacillin) is no longer significant (based on statistical significance analysis using GraphPad Prism biostatistics software; multiple *t*-test analysis was applied assuming equal variances). A synthetic effluent wastewater was designed as a kinetically appropriate reflection of a real wastewater sample, spiked with antibiotics at environmentally relevant concentrations (2 μ g L⁻¹).

The fraction of resistant bacteria in the population after irradiation is depicted in Fig. 1. The concentration of the antibiotic in the effluent matrix is 2 μ g L⁻¹. As the dose increases, the

amount of bacteria continuously decreases. Bacteria are not capable of growing on agar plates after 5 kGy or higher absorbed dose.

Fig. 1 shows that resistant bacteria are significantly more sensitive to radiation treatment over the entire dose range (0.2-2 kGy) than sensitive bacteria when the corresponding antibiotic is present in the matrix. Figure 2 represents the results in the samples without antibiotics.

In this case, the population of bacteria is not affected by low doses. Longer treatment time leads to significant distortions. This is due to the effect of the products formed from the components of the wastewater matrix on bacteria. Comparing the two figures (Fig 1A-B), the resistant bacteria show clearly higher sensitivity towards irradiation. This higher sensitivity does not appear in samples without antibiotics. This leads to the conclusion that that the presence of the antibiotic makes the resistant subtype more sensitive to irradiation. This may be explained by some ongoing biological processes. The sensitive subtype is left intact at very low concentrations of the antibiotic. In contrast, the resistant subtype becomes more depleted, less prepared to the attack of free radicals. In addition, it can be recognized that a higher resistant bacterial fraction was obtained on plates containing piperacillin (Fig. 2B) compared to plates containing erythromycin (Fig. 2A). This observation may suggest that the resistant subtype that survived treatment in the effluent matrix eventually became more suitable for growing on piperacillin-containing plates.



Fig. 1. Fraction of resistant bacteria in the population after advanced oxidation treatment performed directly on a culture containing bacteria, wastewater matrix and either (A) erythromycin or (B) piperacillin at environmentally relevant concentrations (2 μ g L⁻¹).



Fig. 2. Fraction of resistant bacteria in the population after advanced oxidation treatment performed directly on a culture containing bacteria and wastewater matrix without antibiotics. Cell counting was performed on either (A) erythromycin or (B) piperacillin containing plates.

Conclusion

Based on our results, trace level of antibiotics does not bring about an advantage for the resistant bacteria in a mixed resistant / sensitive *Staphylococcus aureus* population. According to our assumptions, it initiates biological processes in the resistant bacteria from which they derive no

benefit. This amount of antibiotic has no effect on the sensitive subtype. Advanced oxidation treatment after optimisation can be a capable technique to eliminate these effects. Nevertheless, we also need to consider that the products from the effluent matrix also have an effect on the bacterial culture. Moreover, antibiotics present at trace amount in the wastewater matrix make resistant bacteria more sensitive towards the advanced oxidation treatment, which might be attributed again to the stimulation of disadvantageous biological processes in the resistant subtype under these conditions prior to the treatment.

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NOVEL INCLUSION COMPLEX OF β-CYCLODEXTRIN AND [2-(2-CHLORO-PHENYLCARBAMOYL)-PHENOXY]-ACETIC ACID ETHYL ESTER

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Abstract

Cyclodextrins (CDs) are cyclicoligosaccharides with 6, 7 or 8 glucose residues joined by a 1-4 glycosidic linkage, produced from starch by enzymatic conversion. Due to the lack of side effects, CDs are FDA-approved compounds for solubilizing, capturing, and delivering lipophilic drugs in humans [1] known for their numerous applications in the pharmaceutical industry, mainly as good carriers for different types of drugs, improving their solubility, stability and bioavailability [2]. Some researches proved that using CDs for encapsulation of poorly soluble active compounds decreases the minimum active concentration required to achieve a biological effect [3] and that the encapsulated compound is released over a significantly longer time period compared with the uncapped one [4].

Salicylanilide derivatives possess antimicrobial, analgesic, anti-inflammatory, hypoglycaemic, antitumoral activities, etc. In order to improve the water solubility, the chemical and physical stability and the drug distribution through biological membranes, a novel complex of [2-(2-chloro-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester with β -CD was obtained using kneading method. The inclusion complex characterization was achieved by modern analytical methods, FTIR, UV-Vis, TG/DSC, X-ray diffraction and SEM analysis. All obtained data confirm the inclusion of the ethyl ester inside the β -CD cavity.

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N-DOPED GRAPHENE QUANTUM DOTS FOR DETECTION OF PALLADIUM(II) IONS AND CARBOFURAN

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Abstract

We produced Graphene Quantum Dots (GQDs) by electrochemical oxidation of graphite rods and exposed them to gamma irradiation at a dose of 200 kGy, in the presence of ethylenediamine. Before irradiation, oxygen was removed from the GQDs dispersion by purging with Ar. These conditions induced both chemical reduction as well as the incorporation of N atoms in the structure of GQDs. Thus, N-doped GQDs were produced. The morphology of dots was investigated using atomic force microscopy. UV-Vis and photoluminescence spectroscopy were used to investigate the optical properties of modified GQDs. The changes in the intensities of PL emission spectra were studied in the presence of different concentrations of Pd²⁺ and pesticide carbofuran. We observed promising results for the application of N-doped GQDs for non-enzymatic PL detection of selected metal ions and molecules of pesticide.

Introduction

Since they have been discovered in 2008. graphene quantum dots (GQDs) attract great scientific attention [1]. Due to their properties, such as dispersibility in water, biocompatibility, chemical stability, emission of photoluminescence in the visible part of the spectrum, resistivity to photobleaching, GQDs have been studied for different applications [2, 3]. In their structure, a small graphene sheet is a core of a dot, while different O-containing functional groups are located at the edges and on the basal plane [4]. Due to a large number of polar functional groups, these dots are dispersible in water and polar organic solvents. The lateral size of GQDs is below 100 nm, while their height varies depending on the number of the graphene layers in the structure, from 0.5 nm for single-layered to a few nm, in the case of multi-layered dots [3]. Due to size, solubility and optical properties, GQDs are investigated for application in bioimaging [5], sensing [6], photodynamic therapy [7], solar cells [8], etc.

There is a large number of methods for GQDs production and they all can be divided into two main groups: *bottom-up* and *top-down*. When dots are produced from small organic molecules, such as glucose or citric acid, these methods are called bottom-up. If GQDs are obtained by oxidative cutting of bulk material based on graphene, these procedures belong to a top-down group. Electrochemical oxidation of graphite rods is a top-down method, powered by electrical current. Due to the electrolysis of the medium, reactive species such as free radicals are produced and they cause oxidation of graphite [9]. From the graphite surface, GQDs were released into the surrounding medium.

Herein we used electrochemical oxidation to produce GQDs and gamma irradiation to modify GQDs structure. As a medium for irradiation, we used a mixture of water, isopropyl alcohol (IPA) and ethylenediamine (EDA). Water was selected because GQDs are highly dispersible, IPA was added due to its ability to efficiently quench oxygen-based free radicals and to assure reductive conditions, while EDA was added as a source of N atoms [10]. The dose of 200 kGy

was applied. These dots were investigated as a sensor for the detection of carbofuran and palladium(II) ions.

Experimental

Electrochemical oxidation of graphite rods was used for GQDs synthesis [9]. GQDs were dispersed in water at a concentration of 1 mg mL⁻¹ and then isopropyl alcohol (3 vol%) and ethylenediamine (4 vol%) were added [10]. Then, the samples were purged with argon for 15 minutes and consequently exposed to gamma irradiation at a dose of 200 kGy at the sterilization facility of the Vinca Institute. This sample is named N-GQDs.

Atomic Force Microscopy (AFM) imaging was conducted using Quesant (Agoura Hills, CA, Unites States) microscope. Tapping mode was chosen, in the air atmosphere. The Q-WM300 rotated, monolithic silicon AFM probe was used. Standard silicon tips (NanoAndMore Gmbh, Wetzlar, Germany) were used with a force constant of 40 N m⁻¹. Diluted GQDs dispersions in MiliQ water in a concentration of 0.125 mg mL⁻¹ were deposited with spin-coated on a mica substrate. AFM images were analyzed using Gwyddion 2.53 software.

UV-Vis spectra were recorded at a Shimadzu UV-2600 UV-Visible spectrophotometer (Shimadzu Corporation, Tokyo, Japan). N-GQD dispersion at a concentration of 0.25 mg mL⁻¹ was measured. Spectra were obtained at room temperature and a normal atmosphere. The selected range was 200-800 nm.

The same dispersion was used for the measurement of PL spectra. They were recorded using Horiba Jobin Yvon Fluoromax-4 spectrometer (Horiba, Kyoto, Japan). The excitation wavelengths were from 300 to 400 nm. The concentration of N-GQD dispersion in water was 0.0065 mg mL⁻¹. Spectra were collected at room temperature in the air environment.

PL spectroscopy was used to investigate the possibility of detection of Pd(II) ions and pesticide carbofuran. First, GQDs were dispersed in the concentration of 0.3 mg mL⁻¹. To this dispersion, the analytes were added. The concentrations of CF were varied from 10 to 100 μ mol L⁻¹ while Pd(II) ions were added 5-200 μ mol L⁻¹. For CF testing, the medium was N-methyl pyrrolidone, while Pd(II) ions water was used as a solvent. After a short incubation time (5 minutes), mixtures of GQDs with analytes were recorded on PL.

Results and discussion

The morphology of GQDs was investigated using AFM (figure 1a and b). As-synthesized GQDs showed an average height of around 1.3 nm and a lateral size between 10 and 30 nm. For the N-GQDs sample, large aggregates can be observed. The average diameter was around 18 nm for p-GQDs, and 15 nm for $_{50\gamma}$ -GQDs.



Figure 1. AFM images of p-GQDs (a, b) and N-GQDs (c, d).
UV-Vis spectrum of N-GQDs showed the main absorption peak at around 204 nm and second, shoulder band at 274 nm (Figure 2a). The band at 204 nm has been assigned to π - π^* transitions in π -domains of GQDs. The second, shoulder band is attributed to π -n transitions of C=O groups. Based on this spectrum it is concluded that N-GQDs have both aromatic π -domains in their structure, but also polar C=O groups were detected too. The previous study showed that these dots also have amino groups in the structure [10]. Other optical properties, such as photoluminescence was studied as well. In figure 2b, PL emission spectra of N-GQDs excited with light of wavelengths from 300 to 400 nm are presented. At different excitation wavelengths, the center of the emission band was changed from 430, 432, 433, 441, 452, 465 nm for excitation at 300, 320, 340, 360, 380 and 400 nm, respectively. This phenomenon is called excitation-dependent photoluminescence and it is associated with a diversity of dots size and chemical composition [11]. The highest intensity of the emission band was detected when the excitation wavelength was 360 nm (figure 2b). Modified dots emit blue light, which is explained by the aromatic domains and sp² core of the dots [12].



Figure 2. UV-Vis (a) and PL spectra (b) of N-GQDs.

The possibility of N-GQDs usage in non-enzymatic PL detection of pesticide carbofuran (CF) and Pd(II), was investigated using PL spectroscopy and these results are presented in figure 3. First, CF was mixed with N-GQDs in concentrations of 10, 50 and 100 μ mol L⁻¹, while the concentration of GQDs was the same (0.3 mg mL⁻¹). As a solvent, N-methyl pyrrolidone was used, due to the insolubility of CF in water. With the increase of CF concentration, the PL intensity was increased as well. These preliminary results indicated that N-GQDs are possible sensor for CF detection, while the mechanism of detection is "turn-on".

In the case of Pd(II) ions, the PL intensity was decreased with the increase of the ion concentrations (figure 3b). These results suggest that PL spectra of N-GQDs are sensitive to the presence of Pd(II) ions, and these changes are particularly emphasized at low ion concentration (5 μ mol L⁻¹).



Figure 3. PL spectra of N-GQDs with diffrent concentrations (in μ mol L⁻¹) of carbofuran (a) and Pd(II) ions (b).

Presented results showed that PL spectra of N-doped GQDs were changed in the presence of CF and Pd(II) ions. It was detected that PL intensity was increased in the presence of CF while ions induced the lowering in the PL intensity. Thus, the future investigation will be deducted to find the concentration range where the linear response between the PL intensity and analyte concentrations is occurring.

Conclusion

In this study, the possibility of the application of GQDs for sensing pesticide carbofuran and Pd(II) ions was explored. GQDs were produced using the ecologically acceptable, electrochemical approach, while gamma irradiation modified their structure. Modified GQDs showed the ability to change their PL intensity in the presence of selected analytes. Namely, the intensity of PL spectra was lowered in the presence of Pd(II) ions and increased when carbofuran was added. These results indicate the ability of N-GQDs to serve as a sensor for selected analytes.

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OPTIMIZATION OF FLOW CONTROL AND CHANNEL PATTERNS DESIGNED FOR USE IN SEQUENTIALLY COUPLED MICROFLUDIC CHIPS AND NANOPARTICLE CHARACTERIZATION BY SINGLE PARTICLE ICP-MS

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Abstract

In this study, we developed polydimethylsiloxane (PDMS) - glass microfluidic chips (MCs) with the ability to function in a sequentially coupled way. We optimized their channel pattern and demonstrated that this feature makes them a more effective tool for carrying out sample preparation steps in single particle inductively coupled plasma mass spectrometry (spICP-MS) such as dilution, counting, and characterization of nanoparticles (NPs).

Introduction

Single particle inductively coupled plasma mass spectrometry is a novel and effective measurement technique, which is used for the characterization of the dispersions of nano- or sub-micron-sized particles. By individually detecting thousands of particles per minute and evaluating the recorded signal profiles, the technique can provide information about the size distribution, number concentration, elemental and isotope composition of the nanoparticles in the sample with great statistical relevance [1, 2]. Another outstanding advantage of spICP-MS is that the particle number concentration that has to be set for the measurement is low $(10^3-10^5 \text{ mL}^{-1})$, thus the sample requirement is very small [2]. In case of solid samples, setting the required NP concentration is no problem, however, the concentration of real-life samples is typically not known, and its adjustment requires a lengthy procedure. As we have shown it in our earlier publications, this task can be automated by utilizing a microfluidic chip as an online sample preparation device interfaced with an ICP-MS instrument [3]. MCs can also be potentially used to automate more complicated sample preparation steps, such as extraction, enrichment, etc. MCs are cost-efficient, easy to produce, compact and versatile devices with the ability to handle low-volume fluid samples. MCs are usually made of polydimethylsiloxane (PDMS) and glass [4]. Despite the MC's great potential in ICP-MS sample preparation, they are not yet widely used. In spICP-MS, one of the obstacles in the way of their use is that the high-level dilution (several orders of magnitude) needed can not be achieved by using a single flow mixer-splitter pattern. For this reason, in this study we developed new MCs that can be used in a sequentially coupled manner, to extend the on-line dilution range of the system.

Experimental

An Agilent 7700X inductively coupled plasma mass spectrometer (ICP-MS) was used in all experiments. Sample introduction was performed through our microfluidic devices in which the flow was generated by utilizing three Gilson Minipuls 3 peristaltic pumps (Gilson Inc., USA). While the outflow was pumped into a MicroMist type nebulizer equipped with a Peltier-cooled Scott spray chamber. The sample uptake rate was 600 μ L/min. The integration time was

set to 500 ms for the measurement of solution samples and 6 ms for nanodispersions, whereas the acquisition time was set to 60 s.

The microfluidic chip molds were fabricated utilizing a Form 3 professional 3D printer using "High Temp" resin (Formlabs, USA). Prior to the molding process, a thin (tridecafluoro-1,1,2,2-tetrahydrooctil)trichlorosilane layer (Gelest Inc., USA) was applied to the mold to facilitate the removal of the cured PDMS from it. The chips were cast using Sylgard 184 silicone elastomer and curing agent (Dow Corning, USA) and sealing the PDMS to a microscope slide with the help of cold oxygen plasma treatment (PDC-002, HarrickPlasma, USA).

Cobalt sample solutions were prepared from 1000 mg/L CertiPUR monoelemental standards (Merck, Germany). In spICP-MS measurements, ultra uniform polyethylene-glycolcapped 47.8 (1.8) nm gold nanospheres were used (Nano-Composix, USA). Trace-quality deionized water from MilliPore Elix 10 device equipped with Synergy polishing unit (Merck, Germany) was used for the preparation of all solutions. Ismatec S3 E-LFL Tygon tubings (IDEX, Germany) with 0.27 or 0.48 mm inner diameter were used for the aspiration of liquid samples. To drive the liquid samples to and from the MCs, stainless steel capillaries with 1.2 mm outer diameter, fabricated from medical needles, were placed in the inlet and outlet ports. For the connection of peristaltic tubing, the inlet and outlet needles and the ICP-MS nebulizer, a PFA tubing with 0.3 inner diameter (Agilent Technologies, USA) and patches prepared from silicone tubing with 1.0 mm inner diameter (Deutsch & Neumann, Germany) were applied. For the inspection of the flow conditions inside the MCs, an Optika Ti600-FL type microscope equipped with a digital camera (Optika, Italy) was used, as well as a Kiralux monochromatic CMOS scientific camera (Thorlabs Inc., USA).

All data processing was performed within the Agilent MassHunter (Agilent Technologies, USA), Origin (OriginLab Corp., USA), and MS Office Excel (Microsoft Corp., USA) software.

Hydrodynamic simulations on flow conditions were performed utilizing the COMSOL Multiphysics software package (COMSOL Inc., USA), in which the models were created utilizing the AutoCAD (Autodesk Inc., CA, USA) and Solid Edge (Siemens PLM Software, , USA) engineering design software.

Results and discussion

For the sequential coupling of the chips, the sample and the diluent flows had to be united and properly mixed, finally the hopefully homogenous flow had to be split into two distinct channels using the same flow rate which was used when the two flows were combined. In an earlier publication of ours we found that the "W" junction is the most effective pattern for combining the flows [3], thus we kept on utilizing this pattern. At the same time, we had to study and optimize the mixing process – the mixing is not cruical if only a single chip is used, because then the ICP-MS spray chamber provides adequate final mixing after nebulization, but in the case of the use of sequentially coupled chips, proper transfer of liquid from chip to chip is only possible if the mixing is good.

For the purposes of mixing pattern optimization, we reproduced four patterns which are commonly used in the literature, each with different operating principles [5, 6, 7]. The mixing in the "serpentine" pattern is strictly based on diffusion, while the "micropillars", "fishbone" and "tesla valve" patterns are designed to cause disturbances in the laminar flow with their protrusions, thus splitting and recombining the flow over and over. To make the mixer designs comparable, we designed them with roughly the same surface area; also, because compactness is a crucial feature in microfluidics. Before the production of the chips, several hydrodynamic simulations were carried out on each design in a search for potential flaws, and to assess their approximate efficiency. Some of the results are shown in Figure 1. and Table 1.



Figure 1. Lateral concentration profiles, plotted for the mid-height plane of the channels (a.) serpentine, b.) micropillar, c.) fishbone, d) Tesla valve)

Mixer design	Serpentine	Micropillars	Fishbones	Tesla Valve	
Number of inlets, their dimensions	3, 700 × 350 μm				
Cross section of outlets	700 × 350, 438 ×350 μm				
Pressure drop [Pa]	2347	742	2451	303	
Internal volume [µL]	105.5	75.0	47.2	35.3	
Concentration difference between the two outflows [mol/m ³]	2.11·10 ⁻⁹	5.99 · 10 ⁻⁸	2.18.10-6	4.74.10-6	

Table 1. Geometric, flow and mixing properties of chip designs

Based on the simulation results, all four designs can complete the mixing process within their length. However, some properties differ for the designs like their flow resistance (closely related to the pressure drop, which, if too high, could burst the microchannels open), and their internal volume (which should be kept as low as possible due to processing speed considerations). In additon to hydrodynamic simulations, we also performed microscope-based imaging experiments using fluorescent dyes. These revealed that the mixing is actually less satisfactory than shown in the simulations, especially in case of the Tesla valve pattern. All in all, the serpentine seemed to be the best mixing geometry, since it provided the highest level of homogeneity at the outlets. Also, its simple geometry (without any constrictions or protrusions), minimizes potential problems associated with the poor wetting properties of PDMS (e.g., bubbles and blockages). These problems were frequently observed with every other chip design, as shown in Figure 2.

In order to optimize the flow splitting process, several experiments were done. We found that if the splitting is done in a passive way, only controlled by the different flow resistances of waste and sample outlet channels with calculated channel cross section ratios, the system shows high sensitivity to random disturbances, thus it was decided to use forced flow (pumping) to be applied. We tested the effect of pumping either the waste or sample outlet ports by gravimetric experiments. Based on five repetitions, an average of 0.67% deviation from the desired flow rate with a 0.81 RSD% was found with the pumping of the waste branch, whereas in the case

of pumping the sample branch, these numbers were much improved, namely 0.01% and 0.4%. Therefore, pumping the sample outlet is more beneficial, which was kind of expected, since the error of the pumping rate are proportional to the flow rate, and the sample outlet flow is always smaller.



Figure 2. Flow imperfections inside a.) micropillar, b.) fishbone, c.) Tesla valve and d.) serpentine channel patterns.

The mixing efficiency was finally determined by diluting a standard 10 ppb Co solution with water. With a tenfold dilution applied, we found only 2.57% deviation from the expected concentration with a relative standard deviation of 2.62% (N= 6). This reflects a very efficient mixing.



Figure 3. Connection diagram of the two coupled chips: a) 60 μ L/min of Au nanoparticle dispersion flow introduced with $4 \cdot 10^5$ mL⁻¹ particle number concentration, b) 270 μ L/min of deionized water flow introduced, c) 60 μ L/min of diluted Au nanodispersion flow transferred into the second chips sample inlet, d) 540 μ L/min of waste flow generated, e) 600 μ L/min of highly diluted nanodispersion flow, which gets introduced to the ICP-MS, X) blocked outlet.

Finally, an experiment was performed on two sequentially coupled serpentine chips, using the arrangement shown in Figure 3. As the sample, a 47.8 nm gold nanodispersion with the number concentration of $4 \cdot 10^5$ mL⁻¹ was introduced, and diluted in the 1 to 225 range, shown on Figure 4. Overall, the number of detection events showed a good linear proportionality with the number concentration of the sample nanodispersion at every dilution rate used. Except in the case of the undiluted sample, when the number of detected particles showed a significant fall-back from the expected value. This difference is caused by the too high particle number concentration, which led to co-detections of particles. Actually, this is the same problem we could face if the measurement of an undiluted nanosuspension would be attempted.



Figure 4. On-line 1 to 225-fold dilution of an Au nanodispersion with 47,8 nm particle diameter and initial particle number concentration of $4 \cdot 10^5 \text{ mL}^{-1}$.

All in all, the chip coupling proved to be a useful and well applicable technique, which extends our capabilities in terms of MC-based on-line nanodispersion preparation. In principle, any number of chips can be sequentially coupled, thereby increasing the maximum achieavable by more than ten times. With 2-4 unit, a 1000-10000-fold dilution can be easily reached, which is fully adequate for most spICP-MS experiments. At the downside, each chip adds to the total channel (dead) volume, resulting in a longer stabilization time and a higher sample volume requirement, thus the pros and cons have to be weighed.

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SUM OF RANKING DIFFERENCES AND GENERALIZED PAIR CORRELATION METHOD IN SELECTION OF OPTIMAL LIPOPHILICITY PARAMETERS OF NOVEL ANTICANCER STEROIDAL DERIVATIVES

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Abstract

Lipophilicity occurs as one of the most experimentally exploited feature of novel potential drug candidates. A novel set of 30 investigated steroids expressed affinity toward different cancer cell lines. In order to additionally characterize these compounds their chromatographic lipophilicity was determined. This study covers chromatographic lipophilicity (log*k*) determination using reversed-phase ultra-high performance liquid chromatography (RP-UHPLC) with polar aprotic and protic solvents and C18 column. As mobile phases mixtures methanol-water (60:40 v/v), methanol-acetonitrile-water (30:30:40 v/v) and acetonitrile-water (60:40 v/v) were used. Also, a set of different *in silico* lipophilicity descriptors (log*P*) and water solubility descriptors (log*S*) were calculated based on 2D and 3D molecular structures, as well as the average log*P* and average log*S* values were estimated. Two non-parametric methods, sum of ranking differences (SRD) and generalized pair correlation method (GPCM) were used for the optimal lipophilicity parameters selection. The experimentally obtained log*k* values and *in silico* log*P* and log*S* values were taken into calculations for SRD and GPCM analysis. Results showed that majority of *in silico* descriptors are placed nearby the experimentally obtained log*k* values and *in silico* lipophilicity estimation.

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EFFECT OF CARBON SOURCES ON XANTHAN BIOSYNTHESIS BY Xanthomonas STRAINS ISOLATED FROM TOBACCO LEAVES

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Abstract

Xanthan or xanthan gum is microbial exopolysaccharide that is produced by aerobic submerged batch cultivation of bacteria of the genus *Xanthomonas* on the appropriate media under optimal conditions. Due to its unique structure and excellent rheological properties, xanthan is commercially the most important biopolymer widely used in different branches of food and non-food industry as well as in agriculture. The success of xanthan production process mostly depends on the metabolic activity of applied *Xanthomonas* strains and the composition of cultivation medium. Among *Xanthomonas* strains that are capable for xanthan biosynthesis, reference strain *Xanthomonas campestris* ATCC 13951 is commonly used in industrial production of this biopolymer. Besides, glucose and sucrose are the most exploited carbon sources in the xanthan production media. However, the rise in prices and the growing demand for mentioned sugars indicate the need for usage of another carbon sources. Although the lack of this idea is difficulty of the reference strain to successfully metabolize most of appropriate carbon sources, the isolation of new *Xanthomonas* strains and selection of adequate substrates are current research topics.

The aim of this study was to evaluate the effect of various carbon sources in cultivation media on xanthan biosynthesis by the reference strain *Xanthomonas campestris* ATCC 13951 and *Xanthomonas* strains isolated from tobacco leaves.

Within the experimental part, the cultivations of reference strain and two isolates (D1 and D2) on semi-synthetic media with fructose, glucose, sucrose, lactose and starch as a carbon sources, respectively, were performed. Xanthan biosynthesis was carried out in aerobic conditions on media with the same initial carbon source content (20 g/L) at temperature of 30° C with agitation (rotary shaker, 250 rpm) for 120 h. The bioprocess success was estimated based on the xanthan concentration and rheological properties of cultivation broths.

The obtained results confirm the possibility of xanthan biosynthesis on all investigated media by all examined *Xanthomonas* strains. In applied experimental conditions, the reference strain was the most productive on media with sucrose (12.62 g/L), glucose (11.03 g/L) and fructose (9.18 g/L), while the lowest product concentration was determined in the media that contained lactose (4.14 g/L) and starch (7.61 g/L). On the other hand, *Xanthomonas* strains D1 and D2 produced xanthan on fructose, glucose, sucrose, lactose and starch containing media in quantity of 1.50 g/L and 0.29 g/L, 0.90 g/L and 1.11 g/L, 1.29 g/l and 4.18 g/L, 5.03 g/L and 4.85 g/L as well as 9.27 g/L and 10.25 g/L, respectively. Comparison of obtained results indicate that strains isolated from tobacco leaves could also represent adequate xanthan producers, whereby lactose and sucrose were considered as the most suitable carbon sources for cultivation of these *Xanthomonas* strains in applied experimental conditions. Results obtained in this research study present a suitable background for future investigations.

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ADSORPTION OF Pb²⁺ IONS ON GAMMA IRRADIATED PLUM POMACE BIOCHAR

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Abstract

Removal of heavy metals is important because of their toxic effects on living organisms and unwanted anthropogenic effects. Biochar is suitable as an adsorbent of heavy metals due to its advantages such as various sources of biomass, a large number of microporous channels and surface functional groups, as well as due to its economic viability. There is no data about the plum pomace biochar usage as adsorbent for lead removal. In this study, the plum pomace biochar modified with gamma irradiation (IrPP) is used for the removal of Pb²⁺ ions from the aqueous solution. The SEM micrographs revealed that surface morphology of plum pomace is suitable for metal adsorption. The results of adsorption kinetics demonstrated that the removal process of Pb²⁺ ions onto IrPP follows a pseudo-second kinetic model, which is confirmed by a better agreement between $q_{e,cal}=227 \text{ mg g}^{-1}$ and $q_{e,exp}=224 \text{ mg g}^{-1}$. Therefore, based on preliminary research, it can be concluded that IrPP originating from biowaste is a promising, eco-friendly sorbent of heavy metal from wastewater.

Introduction

Due to the uncontrolled release of toxic substances from various industrial facilities into the environment, the problem of environmental pollution has arisen. A large number of scientific researches are directed towards the protection and preservation of the environment, and thus of human health.

Biomass as a natural mixture of hydrocarbons is an excellent precursor for obtaining carbon materials. The use of biomass has significant economic and technical advantages. Waste lignocellulosic biomass is used as a raw material for fuel production, heat energy, as well as for adsorption of heavy metals and organic pollutants. In many countries, waste biomass is disposed of in open landfills, which pollutes the environment instead of being used as an energy source or adsorbent [1,2].

In this work, high temperature pyrolysis was used to convert plum pomace into carbon materials.

Plum occupies a leading position in fruit growing, and is mostly grown in the western part of Serbia. About 80% of the total production of plums is processed into brandy. Extensive production of plums, and then processing of plums in order to obtain brandies, increases the amount of waste biomass that is generated as a by-product of processing. Uncontrolled disposal of pomace can pose a great risk to the environment [3].

In this paper, pyrolysis of plum pomace was performed to obtain carbon material that can be used as an adsorbent of heavy metals. Surface modification of obtain carbon material was done by irradiating the material at the source of Co^{60} gamma radiation to improve the adsorption capacity. Scanning electron microscopy (SEM) was used to analyse the morphology of plum pomace before and after irradiation.

The irradiated plum pomace biochar was tested as adsorption materials to remove Pb^{2+} ions. In order to define kinetics and adsorption process equilibrium, corresponding kinetic models were applied.

Experimental

Plum pomace biochar (PP) is a carbon-rich material derived from the oxygenlimited pyrolysis of plum pomace waste biomass. PP were produced using pyrolysis at 500 °C. Modification od PP was done using gamma irradiation of 50 kGy at the Co^{60} source. The surface morphology of the PP before and after radiation (IrPP) was observed by SEM method.

Adsorption experiments

An estimate of the kinetic adsorption parameters of IrPP was executed in a batch of Pb^{2+} solutions. The IrPP dose of 0.025 g was added to 25 mL aliquot of 250 mg L⁻¹ Pb²⁺ solution. The mixtures were shaken at the different times interval (5, 10, 15, 30, 60, 90, 120, 150 and 180 min). The samples were filtered and the residual number of tested metals in filtrates were detected by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES 7400). The adsorption capacity of the Pb²⁺ ions (q_e) were calculated based on the equations (1):

$$q_e(mgg^{-1}) = \frac{(C_o - C_e)V}{m}(1)$$

where C_0 and C_e - the initial and equilibrium concentration (mg L⁻¹), V- the volume of the metal solution (mL) and m-the amount of sorbent (mg).

The adsorption kinetics was simulated with pseudo-first-order and pseudo-second-order models, respectively:

$$ln(q_e - q_t) = lnq_e - k_1 \cdot t \quad (2)$$
$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \quad (3)$$

where q_t (mg g⁻¹) is the amounts of Pb²⁺ adsorbed at time, $k_1(\min^{-1})$ and k_2 (g mg⁻¹ min⁻¹) are the rate constants of pseudo-first and pseudo-second model, respectively.

Results and discussion

The surface morphology of PP and IrPP was examined by SEM analysis. As can be seen in Figure 1, PP shows a smooth surface with a small number of pores and grooves. On the other hand, the presence of pores and the appearance of small cracks on the surface of IrPP are noticed, which is probably a consequence of PP radiation. Petrović et al. [2] concluded that the formation of pores on the surface of biochar can be useful if this material is used as a sorbent of heavy metals from aqueous solutions, because the pores allow easier internal diffusion of metal ions from wastewater.



Fig. 1. SEM of PP (a) before and (b) after radiation.

Adsorption kinetics

The adsorption kinetics of Pb^{2+} ions by IrPP were presented in Figure 2 and the kinetics parameters were summarized in Table 1.



Fig. 2. Pseudo-first-order (a) and pseudo-second-order (a) kinetic adsorption curves of IrPP for Pb^{2+} ions

Although both models described the kinetic data well with all R^2 higher than 0.99, the pseudosecond-order model gave better fits (R^2 =0.9999) and compared to pseudo-first-order kinetic model (R^2 =0.9933), suggesting that the adsorption of Pb²⁺ by the PP was ascribed to chemical process [4]. Additionally, this was verified with the better agreement of theoretical ($q_{e,cal}$) and experimental results ($q_{e,exp}$) for the pseudo-second model.

Table 1 The kinetic parameters of Pb ²⁺	ions adsorption using IrPP
	Contaminant
	Pb^{2+}
$q_{eq,exp} (mg g^{-1})$	224
Pseudo-first-order model	
$q_{eq,cal} (mg g^{-1})$	66
$k_1 (min^{-1})$	0.124385
\mathbb{R}^2	0.9933
Pseudo-second-order model	
$q_{eq,cal} (mg g^{-1})$	227
$k_2 (g mg^{-1} min^{-1})$	$8.58 \cdot 10^{-08}$
R ²	0.9999

Conclusion

Based on preliminary research, the SEM analysis demonstrated that radiation significantly changed the structure of the PP. That is, a large number of pores were observed on the surface of PP after radiation, which facilitate the internal diffusion of Pb^{2+} from aqueous solutions. Also, on the basis of kinetic analysis, it was confirmed that Pb^{2+} adsorption is well described by a pseudo-second order kinetic model. These results show that IrPP can be used as a cost-effective sorbent of heavy metals from wastewater.

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THE MORPHOLOGY OF PANI/GRAPHENE COMPOSITES PREPARED UNDER ISOTHERMAL CONDITIONS

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Abstract

This article investigates the morphology of polyaniline/electrochemically exfoliated graphene composites prepared by oxidative polymerization with ammonium persulfate at a constant temperature (15 °C). During the polymerization, the pH of the reaction mixture decreases, which was used to monitor the rate of the reaction. A granular morphology with the presence of large sheet-like structures is found to be predominant for the composite prepared without added acid, while rod-shaped and tubular morphology is characteristic for the composite prepared with the addition of glacial acetic acid. The nanotubular and nanorod morphology of the sample prepared with the addition of glacial acetic acid was also confirmed by the presence of prominent phenazine structural units in Raman spectra. The composite prepared with the addition of HCl has granular morphology with a small amount of rod-like structures.

Introduction

Polyaniline (PANI), as a member of the intrinsically conducting polymers family, is one of the most studied polymers because of its facile synthesis, environmental stability, low cost, and reversible acid-base doping-dedoping process which influences its electrical conductivity [1, 2]. Due to its electrical, optical, and electrochemical properties, PANI is considered a suitable material for various applications such as counter electrodes in dye-sensitized solar cells [3], electrode material for supercapacitors [4], sensors [5], and corrosion protection films [6] among others. Among the three forms of PANI (leucoemeraldine, emeraldine, and pernigraniline) only the emeraldine salt is conductive due to the conjugated p-electron system. Numerous studies have shown that doping PANI with various dopants could significantly improve the conductivity of PANI [7-9]. Recently, composites of PANI and graphite, graphene oxide (GO), or reduced graphene oxide (rGO) are extensively studied for boosting the capacitance of supercapacitor devices [10], enhancing the dispersive and crystalline performance of corrosion protection films [11, 12], and improving the thermal stability of the nanocomposite [13]. Graphite has a layered structure composed of sp^2 hybridized carbon atoms arranged in a honeycomb structure. Individual layers of graphite known as graphene have attracted considerable interest as a filler in polymers due to its electrical, mechanical, and thermal properties [14-16]. However, graphene as a filler is mostly present in its highly oxidized form as a GO with a diminished electrical conductivity. One way to overcome this issue is to partially restore graphene's structure by reduction to obtain rGO, but its conductivity remains incomparable to the graphene's. An alternative pathway is electrochemical exfoliation of graphite to obtain graphene with preserved structure and electrical properties [17-19]. During the process, produced hydroxyl ions weaken van der Waals forces between graphite layers which allows the intercalation of electrolyte ions that form gas bubbles upon reduction which are responsible for graphene exfoliation. In this way it is possible to obtain single- and fewlayer graphene with a large flake size and well-preserved structure, but with a sufficient amount of polar groups attached to graphene to make it water dispersible [20].

The goal of the present work was to investigate the morphology of PANI/graphene composites. The samples were prepared under isothermal conditions in the presence of electrochemically exfoliated graphene. The change of pH during polymerization was monitored and the samples were characterized by SEM and Raman spectroscopy.

Experimental

Electrochemically exfoliated Highly Oriented Pyrolytic Graphite (eHOPG) was prepared as described previously [17]. Solution 1 was prepared by mixing 10 mg of eHOPG and 1 ml of aniline (Sigma Aldrich) in 49 ml of DI water until well homogenized. Solution 2 was prepared by dissolving 2.85 g of ammonium persulfate (Fluka) in 50 ml of DI water. The polymerization reaction was conducted in EasyMax 102 Basic Thermostat System by mixing solutions 1 and 2 at a constant temperature of 15 °C under stirring (100 rpm). The change in pH was monitored during the reaction (Mettler Toledo pH meter). The obtained product was centrifuged three times at $2575 \times g$ for 30 minutes and the supernatant was discarded. Finally, the precipitate was dried in a vacuum oven at 60 °C overnight. Samples PANI-HCl and PANI-CH₃COOH were prepared following the same procedure with the addition of 2 ml 0.2 M HCl (Sigma Aldrich) or 2.3 ml of glacial acetic acid (Merck) in the reaction mixture, respectively.

SEM analysis was performed on a Zeiss Supra 35 VP field-emission electron microscope at an accelerating voltage of 1 kV. Small amount of each sample was mounted on aluminum studs using adhesive graphite tape. Raman spectra were recorded by confocal Raman microscope (alpha 300 R+, WiTec, Ulm, Germany). The excitation laser of the 532 nm wavelength and 0.22 mW power was focused using $100 \times (NA = 0.9)$ objective onto the sample surface. The confocal pinhole diameter was 50 µm and the accumulation time for a single Raman spectrum was set to 40 s. Constant background was subtracted from all measured Raman spectra.

Results and discussion

Polymerization of aniline was performed in the presence of electrochemically exfoliated graphene under a constant temperature of 15 °C. It was shown that under lower temperatures the synthesized PANI shows better physicochemical characteristics [21], however, if the temperature is too low the polymerization takes a longer time to complete. During the polymerization the change of pH of the reaction mixture was monitored (Figure 1).



Figure 1. The changes of pH of the reaction mixture during time for PANI/ graphene composites.

The generation of protons during the polymerization leads to the decrease of pH of the reaction mixture. An initial fast decrease in pH is induced by the fast oligomerization of aniline molecules, which is followed by a slower pH decrease due to the prevalence of less-oxidizable anilinium cations and the decrease of the concentration of ammonium persulfate [22]. The next step is characterized by the rapid pH decrease caused by polymer propagation. In this phase, pernigraniline-like oligoaniline units have sufficient power to oxidize both anilinium cations and leucoemeraldine- and protoemeraldine-like oligoanilines, and parallel to this pernigraniline-like oligoanilines become oxidized by remaining peroxydisulfate. These polymerization steps are clearly distinguished for all three samples, however, the rate of the polymerization is much slower for the PANI-CH₃COOH sample compared to PANI and PANI-HCl samples. Also, the final pH value for PANI-CH₃COOH sample is slightly higher than the final pH value of PANI and PANI-HCl samples (1.18 compared to 0.78 and 0.85, respectively).



Figure 2. SEM images of PANI/graphene samples: a) PANI, b) PANI-HCl, and c) PANI-CH₃COOH.

According to SEM images (Figure 2), PANI/graphene composite synthesized without added acid has granular morphology with the presence of large sheet-like structures. Sample PANI-HCl is morphologically similar to sample PANI, but a certain amount of rod-like shapes can be detected. On the other hand, sample PANI-CH₃COOH is dominated by rod- and tube-like structures with diameters between 100 and 150 nm and lengths of up to 1 μ m. Besides, a small amount of irregular shapes and granular particles can also be detected.



Figure 3. Raman spectra of PANI/graphene composites.

In Figure 3 Raman spectra of PANI/graphene composites are shown. Typical D and G Raman bands of graphene that appear at 1351 and 1591 cm⁻¹ are strongly overlapped by bands that are associated with PANI structural units: band at 1586 cm⁻¹ originates from the C=C and C-C stretching vibrations of the quinoid rings, while the band at 1344 cm⁻¹ stems from the C-N⁺⁺

stretching vibrations of delocalized polaronic structure [23]. Besides, in the spectrum of PANI/graphene prepared in the absence of acid, bands at 1410 and 1394 cm⁻¹ that arise from the substituted phenazine structural units in polymer structure are also observed. Except for the aforementioned bands that stem from PANI units, spectra of PANI-HCl and PANI-CH₃COOH contain bands at 1180 cm⁻¹ (the C-H bending in-plane vibration of semiquinonoid rings), 1234 and 1249 cm⁻¹ (the C-N stretching in benzenoid units), 1561 and 1392 cm⁻¹ (stretching of substituted phenazine structural units), and 1631 cm⁻¹ (C-C/C=C stretching vibrations of benzenoid units with a contribution of the substituted phenazine units). Phenazine structural units indicate the presence of nanotubular and nanorod PANI morphology [24]. As expected, the bands that correspond to phenazine units are most prominent in PANI-CH₃COOH spectra.

Conclusion

PANI/graphene composites were prepared by oxidative polymerization of aniline with ammonium persulfate at a constant temperature (15 °C). Additional samples were prepared with the addition of 0.2 M HCl, or glacial acetic acid. Polymerization rate was monitored by the change of pH of the reaction mixture. Comparable polymerization rates were measured for PANI and PANI-HCl samples, while the reaction with added acetic acid takes longer to complete. Granular morphology with large sheet-like structures was found for PANI sample. PANI-HCl sample has similar morphology as PANI, but with a presence of a certain amount of rod-like shapes. Sample PANI-CH₃COOH is dominated by rod- and tube-like structures with diameters between 100 and 150 nm and lengths of up to 1 μ m. Nanotubular and nanorod morphology of PANI-CH₃COOH sample was confirmed by the presence of prominent phenazine structural units in Raman spectra.

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CATALYTIC REACTION OF CARBON DIOXIDE WITH METHANE ON SUPPORTED Co/Mo CATALYSTS

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Abstract

In this work the reforming of methane with carbon dioxide has been investigated at 773 K on supported Mo-Co/ Al_2O_3 catalysts with different loading 1%, 5% and 10 %. of Mo. The catalysts were characterized by XRD and TEM methods before and after the reaction. The results revealed that 10%Co/ Al_2O_3 had the highest conversion of CH₄ and CO₂ and highest syngas formation rate among the mentioned catalysts. In addition, the mentioned catalysts characterization before and after the reaction showed no differences.

Introduction

Some carbon-containing fossil fuel combustions, such as coal, natural (stranded or shale) gas, and petroleum, have emitted significant amounts of greenhouse gases, which can cause global climate change as well as environmentally hazardous pollutant emissions. [1]. Renewable energy development and greenhouse gas emissions reduction have become increasingly crucial global issues in recent years [2]. The conversion of CH_4 and CO_2 , the two primary components of biogas and the principal greenhouse gases into synthesis gas is in the focus of academic and industrial research. Reacting the CH_4 and CO_2 is one option for achieving this goal [3]. The CH_4 can react with CO2 as a reductant - by the techniques including Dry reforming (DR), Steam Reforming (SR) and Combining SR and DR -to produce the industrially important syngas (CO+H₂ mixture) [4]. From the CO+H₂ mixture both fuel and methanol or a variety of other products can be produced using currently available technologies [5].

Experimental

In this research, the DR reforming of methane with carbon dioxide has been investigated atmospheric pressure, at 773 K, using 10%Co/Al₂O₃, 1% Mo+10% Co/Al₂O₃, 5% Mo+10% Co/Al₂O₃ and 10% Mo+10% Co/Al₂O₃ catalysts, which were prepared by incipient wetness method and calcined at 773 K. The experiments were carried out in a fixed-bed continuous flow reactor. The amount of the catalysts used were 150 mg. The flow rate of the reactants was 30 ml/min. The catalysts were oxidized for 30 minutes and reduced for 60 minutes at 773 K and the catalytic tests were carried out at 573- 873K. The catalysts were characterized by XRD and TEM methods before and after the reaction.



Gas Chromatograph (Agilent 6890)



MiniFlex II DESKTOP X-ray DIFFRACTOMETER (Rigaku)



FEI TECNAI G2 20 X-Twin high-resolution transmission electron microscope (HR-TEM)

Results and discussion

The results indicate that 10%Co/Al₂O₃ had the highest conversion of CH₄ and CO₂ and highest syngas formation rate among the mentioned catalysts. Furthermore, Mo promoted 10%Co/Al₂O₃ was found to be more active in 1% amount than the 5% or 10% Mo/Al₂O₃.



Fig.1. CO₂ and CH₄ Conversion in heating phase



Fig2.CO₂ and CH₄ Conversion in isotherm phase





Fig.3.CO/H₂ in heating phase and isotherm phase





Fig.5. TEM pictures & XRD graph

Conclusion

The 10%Co/Al₂O₃ reforming catalyst had the best performance in terms of CH₄ and CO₂ Conversion. In addition, among the promoted catalysts by Mo, 1% Mo+10%Co/Al₂O₃ was more active (Fig.1-2.)

- ➤ The main products of the reaction were H₂ and CO. The 10%Co+1%Mo/Al₂O₃ showed the highest ratio of CO/H₂ in heating phase, while the 5%Mo+10%Co/Al₂O₃ showed the highest ratio in isotherm phase. (Fig.3.)
- The 10%Mo+10%Co/Al₂O₃ had the fast-increasing CO/H₂ ratio in the heating phase and had the steady trend in the isotherm phase. (Fig.3.)
- The catalysts were characterized before and after the reaction with XRD and TEM which no differences were found before and after the reaction (Fig.5.).

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THERMAL KINETIC ANALYSIS OF THE SPENT MUSHROOM SUBSTRATE AND HYDROCHAR

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Abstract

A carbon-rich product, hydrochar, was synthesized by hydrothermal carbonization (HTC) of spent mushroom substrate (SMS), at temperature of 260 °C. The thermal kinetic analysis has shown that hydrothermally treatment improve the combustion behavior of hydrochar. The kinetic parameters were determined by Kissinger and Ozawa methods. The SMS-260 had a significantly lower activation energy compared to the SMS, which means that this hydrochar needs a smaller amount of energy to start combustion. Generally, the preliminary results show that HTC is an effective way to transform SMS into alternative solid biofuel.

Introduction

The growing world population and industrial development require more energy than can be supplied by conventional means [1]. Excessive use of fossil fuels is accompanied by an increase in emissions of carbon dioxide, nitrogen oxides and other harmful particles, which has negative consequences for the environment [2]. Therefore, in order to reduce the problem of this kind, the increasing focus is on the use of biomass as a renewable energy source. The advantage of biomass is easy transformation into another form of energy, using various thermochemical processes. HTC compared to other thermochemical methods has shown numerous advantages such as lower reaction temperatures, shorter process duration, the possibility of using biomass without prior drying, low ash content in the obtained products, prevention of CO_2 and other harmful gases during biomass processing, etc [3].

The conversion of biomass to a hydrochar using HTC takes place in an aqueous medium, at a moderate temperature (180–260 °C) and autogenous pressure (2–10 MPa) [4]. The properties of the hydrochar are regulated by process parameters (pressure, temperature, reaction time, and biomass and water ratio) [4]. Depending upon the process conditions used, the hydrochars can be formed with significantly improved fuel characteristics.

In addition, the data obtained from the thermal kinetics of hydrochar can be of great importance for the design of combustion equipment on an industrial scale [5,6]. Therefore, in this study, SMS from Agaricus bisporus production was carbonized using HTC technology at 260 °C and then kinetic anlysis was carried out of SMS and hydrochar using TG data at three heating rates.

Experimental

Material and synthesis of hydrochar

SMS was obtained from the local mushroom production Ekofungi, located in Belgrade. Synthesis of hydrochar were performed in a autoclave reactor (Carl Roth, Model II). About 10 g of SMS was mixed with 150 mL of deionized water and heated at 260 °C for 60 min. After that, the reactor was cooled down to room temperature. The obtained hydrochar denoted as SMS-260.

Thermogravimetric analysis

The thermal properties of the SMS and SMS-260 were investigated by the simultaneous nonisothermal TGA and DTA using a Setaram Setsys Evolution 1750 instrument. The samples were heated from 25 to 1000 °C with a heating rate of $\beta = 5$, 10 and 20 °C min⁻¹ in the air atmosphere. The average mass of the samples was about 7 mg.

The relevant kinetic parameter, activation energy (E_a) , in the combustion process can be obtained by the Kisinger (1) and Ozawa (2) method, which is based on the functional dependence of the heating rate (β) and the exothermic peak temperature shift (T_{max}):

$$\log\left(\frac{\beta}{T_{\max}^{2}}\right) = \log\frac{Z \cdot R}{Ea} - \frac{Ea}{2.303 \cdot RT_{\max}} (1)$$

$$\log\beta = \log\frac{z \cdot Ea}{R} - 2.315 - 0.4567(\frac{Ea}{R \cdot T_{max}})$$
(2)

According to the Eq. (1) and Eq. (2), the plot of $\log\left(\frac{\beta}{T_{max}^2}\right)$ or $\log\beta$ against $1/T_{max}$ will present a straight line at different heating rates. Then kinetic parameters can be derived from the plot. The activation energy E_a can be calculated from the slope while the pre-exponential factor Z is determined by the intercept of regression lines.

Results and discussion

Kinetic parameters of thermal degradation are required for effective prediction of combustion behavior and optimization of the thermal decomposition of the different feedstock. The crucial kinetic parameters, activation energy (E_a), pre-exponential factor (Z) and correlation coefficient (R^2) at various heating rates of SMS and SMS-260 were designated by the Kisinger and Ozawa.

Results from Table 1, showed that SMS-260 has better combustion properties that the SMS, visible through the lower E_a [2]

The Ea values for SMS were 238.8 and 235.7 kJ mol⁻¹, while for SMS-260 were 53.4 and 61.7 kJ mol⁻¹ calculated by Kissinger and Ozawa methods, respectively. The R² obtained by the Ozawa was higher than that obtained by the Kissinger, indicating that the Ozawa method was more suitable for the evaluation of the combustion process of samples.

The values of the pre-exponential factor (Z) show the existence of the complex nature of the material and the reactions that take place during combustion. The Z value, less than 10^5 s^{-1} is ascribed to porous structures and suggesting easier and faster decomposition of SMS-260 [7]. On the other hand, the values of Z of more than 10^{14} s^{-1} indicate a slower and difficult decomposition of the SMS structure [7].

Kinetic parameters of SMS and SMS-260 by Kissinger and Ozawa.						
	Kissinger		Ozawa			
Samples	E _a (kJ mol ⁻¹)	\mathbb{R}^2	$Z(min^{-1})$	E _a (kJ mol ⁻¹)	\mathbb{R}^2	$Z(min^{-1})$
SMS	238.3	0.9912	$3.84 \cdot 10^{21}$	235.7	0.9918	$2.38 \cdot 10^{21}$
SMS-260	53.4	0.8950	$1.71 \cdot 10^3$	61.7	0.9294	2.59·10 ³

Conclusion

Table 1

Based on the kinetic parameters, it can be concluded that HTC has improved the combustion property of the SMS. A comparative study of the thermal combustion kinetics of SMS and

SMS-260 at different heating rates was conducted using the Kissinger and Ozawa methods. The Ozawa method has proven to be more suitable for assessing the combustion process of samples. The SMS-260 has shown lower activation energy compared to SMS, showing that HTC technology is efficient for converting SMS into solid biofuel with improved combustion performance and reactivity.

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PREPARATION OF *N*-HETEROCYC COMPOUNDS BY ENVITONMENTALLY BENIGN CASCADE REACTIONS

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Abstract

The *N*-heterocyclic compounds include various molecules with significant biological activity, such as the quinazolinone derivatives. The preparation, characterization and application of these molecules are in the focus of pharmaceutical chemistry. The mono- and disubstitued dihidroquinazolinone derivatives are potential anti-cancer and anti-inflammatory compounds, besides they can be used as other pharmaceutical and agrochemical ingredients [1]. Nowadays the demand for the protection of the environment is fortunately increasing, which places numerous expectation on the industries. In the fine chemical and pharmaceutical processes, it has become a basic requirement to take into account the 12 principles of green chemistry. Due to this, the catalytic and neat reactions along with the application of compound from sustainable sources, environmentally friendly solvents and alternative activation methods have a great importance. Among the alternative energy transmission methods, it is worth to highlight the implementation of the mechanochemistry, as the reactions carried out in ball mills, have many of the above-mentioned advantages.

In this present study, we examined the cascade reaction of anthranilamide and acetone, in which one ring-closing step occurs, and then we expanded the system to a bifunctional compound, ethyl levulinate, which is a renewable reaction partner, to achieve a second ring-closing step. We examined the possibilities of neat reactions, the quality of the applied catalyst, reaction temperature and time. After the successful optimization of the magnetically stirred neat reactions, we turned our focus to the mechanochemical activation of the reactions. It is possible to carry out several reactions neat in a ball mill, however generally better result can be achieved by adding a small amount of solvent to improve the mixing and the energy transition in the system [2]. Under the optimized conditions, the reactions were carried out with outstanding selectivity, and the final products were obtained in good yields.

The developed method provides essential experiences to synthesize optically pure *N*-heterocycles in a system that meets today's environmental expectations. The initial experiment of the reaction between anthranilamide and other bifunctional compounds was also carried out. It is our ongoing future goal to optimize the conventional, then the mechanochemical synthesis of optically pure condensed ring systems.

Introduction

Numerous methods are known for the synthesis of *N*-heterocycles, such as the aza-Wittig reaction of *o*-amino benzoic acid or anthranilamide and carbonyl compounds or esters [3]. The cascade reaction can be carried out in a Au(I)-catalysed one-pot system [4]. The reaction of anthranilamide with aldehydes and ketones starts with the formation of a Schiff-base, which cannot be isolated, then follows the ring-closing step. As long as the reactant of anthranilamide is bifunctional and bears the necessary structural characteristics, such as levulinic acid or its esters, the tandem reactions is continued with another ring closing [5]. To catalyse this latter step various homogenous and heterogeneous catalysts, for example Bronsted-acid are available. Although, these latter methods are successful, their sustainability should be increased. Various

alternative activation methods can be used, such as microwave and ultrasound assisted or mechanochemical reactions. Many advantages of the latter can be highlighted, starting from minimized solvent need to significantly shorter reaction times.

Due to the fast movement of the mixing mills, the collision and friction between the grinding media, the wall of the mixing jar and the components provide enough energy to activate various reactions. In most of the mechanochemical systems, the reactions are carried out neat. However, a small amount of liquid added to the system acts as an energy mediator, thus the term liquid assisted grinding (LAG) is used to describe these methods [2]. The application of mechanical energy transfer opens a path to carry our various catalytic reactions faster, than the conventional, thermally activated reactions [6,7].

Our aim was to develop an environmentally benign method for the synthesis of *N*-heterocycles, in the reaction of anthranilamide with acetone and bifunctional molecules, to achieve a second ring closing step in a one-pot cascade manner. As the reactant in the latter method ethyl levulinate was chosen, due to its sustainable properties, as the platform molecule, levulinic acid is obtained from renewable biomass sources, furthermore the esterification may be carried out in a neat and microwave activated organocatalytic system [8,9].

Experimental

The anthranilamide, acetone, ethyl levulinate and the catalyst (Amberlyst 15) were, obtained from commercial sources and were used as received. The conventional magnetically stirred cascade reactions were carried out in closed glass vials, which were immersed in heated oil bath if it was necessary. After the given reaction time the mixture was cooled to room temperature than 2 mL methanol (MeOH) was added to dissolve the product. The Amberlyst 15 beads were separated from the solution and were washed twice with 1 mL MeOH. In the mixing mill, during the reaction the Amberlyst 15 grinds to powder. To make the stirred and milled reactions comparable, the catalyst was pre-grinded at 30 Hz for 10 minutes, to obtain a homogenous powder. 2 mL MeOH was added to the reactions carried out with Amberlyst 15 powder, the catalyst was separated by centrifugation and was washed twice as before. The combined organic phases were analysed by GC-MSD and GC-FID.

The mechanochemical reactions were carried in 10 mL ZrO₂ grinding jars with milling balls made from the same material with the diameter of 5 mm. In a typical run all the compounds were measured into to the jars, then the necessary amount of balls was added into the system. The mixture then was agitated in a Retsch Mixing Mill MM 400 instrument at the desired frequency for the desired time. When the reaction was completed 2 mL MeOH was added, then the jars and balls were washed twice with 1 mL MeOH. The catalyst was separated by centrifugation, then was washed twice as in the case of the stirred reactions. The final product was purified by crystallisation from hexane and was analysed by NMR spectroscopy.

Results and discussion

Our first experiments were carried out in a conventional magnetically stirred system. The twostep reaction between anthranilamide and acetone was carried out neat, acetone acted as the solvent as it was introduced to the system in excess. The formation of the Schiff-base is fast, however, the ring-closing step demands a catalyst. The application of Amberlyst 15 was efficient to achieve closely full conversion, and only the expected compound with the condensed ring system was detected as the product.



Scheme 1. The reaction of anthranilamide and acetone with and without catalyst (24°C, 6h)

The one pot cascade reaction of anthranilamide and ethyl levulinate has similar first two steps as the Schiff-base is formed, followed by the first ring closing, although due to the bifunctional characteristics of the ketoester a second ring closing can occur. Similarly to the latter reaction, the reactant was added in excess (1.5 eq) to act as a solvent and to ensure the proper mixing of the system. The reaction was carried out at 60°C, and was stirred for 24 h.



Scheme 2. The reaction of anthranilamide and ethyl levulinate (60°C, 24h)

We examined the effect of the amount of catalyst first with the commercially available Amberlyst 15 beads. In this reaction the conversion was closely full, the amount of catalyst had no effect on this parameter, however the selectivities changed drastically. Figure 3 shows the selectivity of B as a function of the amount of catalyst, which gives a saturation curve. The prepowdered catalyst is more effective, probably due to the larger surface, better accessibility of the catalytic sites and more efficient distribution in the system. The main difference between the original and pre-grinded Amberlyst 15 is detectable when 50 mg of catalyst was applied. The conversion was the same, however the second ring-closing step was faster, when the powdered catalyst was applied. The magnetically stirred reaction was also carried out in the presence of an additional solvent, in our case, MeOH. To determine de amount of the added liquid, we choose to reduce the excess of the levulinate to 1.1 eq, and the missing volume (57 μ L) was supplemented with MeOH. The effect of the solvent was examined in a 24 h reaction applying 50 mg powdered Amberlyst 15. As Figure 1. shows, a small amount of solvent improved the reaction, to reach almost full selectivity of the final, tricyclic product (**B**).



Figure 1. The selectivity of the reaction of anthranilamide and ethyl levulinate in batch reactor with the use of ●-commercially available and ■-pre-grinded Amberlyst 15, ◆-in the presence of MeOH

The next step was to examine the mechanochemical implementation of the cascade reaction between anthranilamide and ethyl levulinate. In a typical run 35 pieces of grinding balls with a diameter of 5 mm were applied, to provide enough collisions. As the Amberlyst 15 beads would have been grinded during the milling, in order to make the system reproducible, Amberlyst 15 powder was applied. The main advantage of the mechanochemical processes is that the reaction time can be significantly shortened compared to the thermally activated systems. We were able to shorten the 24 h reactions to only 3 h.

In the LAG method a small amount of liquid is necessary, which has an energy mediating role, besides it provides better mixing. The effect of the amount of MeOH is shown on Figure 2. The achieved result illustrates the energy mediating role of the liquid. The energy provided by the friction and collision of the grinding media is well distributed and transferred by the additional liquid. Furthermore, the added MeOH dissolves the compounds and helps to mix evenly with the catalyst on the surface of the balls. Although the reaction is not neat literally, LAG methods are still classified as environmentally friendly processes and MeOH is considered a green solvent.



Figure 2. The effect of the amount of applied MeOH on the conversion and selectivity; -Conversion; -Selectivity B

The milling time did not affect the conversion, due to the fast formation of the Schiffbase and the first ring-closing, however, the second ring-closing step is time consuming. The reaction was milled for 60, 90, 120 and 180 minutes, and the ratio of the product changed significantly. After 60 minutes only 41% **B** was detected, which increased to 71% and 75% after 90 and 120 minutes of grinding. To obtain the product after the second ring-closing in >99% at least 180 minutes were necessary at 30 Hz agitation frequency.

The amount of the transferred energy depends on the collision intensity, which is affected by the mass of the grinding balls [10] and the milling frequency. As the agitation was slower (20 Hz) the selectivity of **B** decreased from >99% to 18%, which shows the high activation energy of the second ring-closing step. The reaction was agitated at 30 Hz for 3 h, using different grinding balls. In the cases of the smaller milling media sizes (Ø3 and Ø5 mm) the number of balls were determined to apply similar total ball volumes and only 1 pc was added from the bigger (Ø12 and Ø15 mm) balls. Although the 125 pcs of the Ø3 mm beads provided enough collisions per unit of time, the kinetic energy of the light weighted balls, thus the intensity of the collisions was not enough to promote the second ring-closing. Figure 3. shows the importance of the intensity of collisions as less pcs of the Ø5 provided outstanding selectivity. In the case of the Ø12 and Ø15 mm balls, the mass, thus the energy of the grinding media was high, however due to the lack of collision points, the selectivity decreased drastically.



Figure 2. The effect of the applied balls on the selectivity

The reactions in both the magnetically stirred and the mechanochemical systems were carried out applying 1 mmol anthranilamide. The reactions were successful, and provided enough product to purify by crystallisation, thus, the method opens a new path for an environmentally benign synthesis of *N*-heterocyclic compounds.

Conclusion

In summary, we developed a sustainable system for the synthesis of *N*-heterocycles. The reactions were carried out neat in glass vials, and by a LAG method using a mixing mill. The mechanochemical implementation bears many advantages over the conventional magnetically stirred methods. The reaction time was shortened from 24 h to only 3 h, while the achieved conversion and selectivity remained the same. As the LAG method demands a small amount of additional liquid we aimed to apply a solvent which dissolves the starting compounds and the products, but most importantly complies with the principles of green chemistry. A small amount of methanol provided better mixing and had an energy mediating role, thus the mechanochemical reaction could be completed. The final product was separated from the heterogeneous catalyst by dissolving it in methanol, and then after evaporation, could be purified by crystallisation.

Our studies are the first steps to develop a sustainable method for even chiral *N*-heterocycles in the future. Furthermore, as a heterogeneous catalyst is applied, the possibility of reusing the catalyst may further improve the sustainability of the method.

Acknowledgements

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SEPARATION OF VALUABLE ENDOGENOUS COMPONENTS FROM CARROT PEEL BY MICROWAVE EXTRACTION

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Abstract

With the rapid development of food technology, the amount of by-products generated is proportionally increasing as well. If not reused, by-products can pose a serious problem to our environment. In addition to managing and reusing such by-products, they may be valorised by extraction of valuable endogenous components using a less sophisticated, plain but modern technology such as microwave-assisted extraction. This new technique has been gaining popularity in recent years because compared to conventional methods, microwave-assisted extraction is characterised by shorter extraction times and lower solvent requirements.

This preliminary experiment aimed to demonstrate that with the right operating parameters and solvent ratio, this method can be used for the efficient extraction of valuable endogenous components from carrot peels. To quantify the endogenous components, polyphenol content (TPC), antioxidant capacity (FRAP) and carotenoid content were investigated. The findings of this study showed that the optimal valuable substance extraction was obtained at 1:10 peel-solvent ratio at 100 W microwave power for fresh carrot peels, and 1:20 peel-solvent ratio at 800 W microwave power for dried carrot peels.

Introduction

According to the ever-increasing demands of the population, food production is rising worldwide. As the amount of the produced food increases, so does the amount of "waste" or by-products generated by the technological processes. As such, carrot processing creates a significant amount (20-25%) of peel waste, which causes environmental and recyclability problems for the food industry. [1-2].

Carrot peel waste contains/may contain large amounts of bioactive substances, which are yet given little commercial value. However, these phytochemicals could be profitably exploited as natural additives either in pharmaceutical or food industry, in agriculture, or in other areas. [3].

Carotenoids and polyphenols are possibly the most valuable components of carrot roots. Both have antioxidant activity, protect the body against cardiovascular disease and cancer, and have anti-inflammatory and immune-boosting effects [4]. Extraction is a suitable method of obtaining these valuable components. In addition to conventional extraction methods, new extraction techniques emerge nowadays, such as Disposable Pipette Extraction (DPX) [5], Ultrasound-assisted Solid-liquid Extraction, Micro Extraction by Packed Sorbent (MEPS) [6], and Microwave-assisted Extraction used in the presented experiment. [7].

Experimental

Materials and solvents

The raw material used was the peels of Nanti carrots (Daucus carota L. ssp. sativus), commercially available in Hungary. Distilled water and 20% ethanol were used as extractants. All chemicals were purchased from Sigma Aldrich.

Sample preparation

After cleaning and weighing the carrots, they were peeled. Then, the about 1 mm thick peels' weight was measured. The carrot peels prepared for drying were dried to constant weight in the drier at 60°C temperature. Afterwards, the fresh and the dried carrot peels were shredded using a laboratory shredder.

For the extraction, 10 ml of solvent were added to 1 g of fresh carrot peel and 20 ml of solvent to 1 g of dried carrot peel.

The extraction

To extract the endogenous components (polyphenols and carotenoids), a plain but also modern technology was chosen: microwave-assisted extraction. To prepare the analytical testing, three different solvents and three different microwave power levels (100 W, 450 W and 800 W) were used for the extraction. The extraction time was 30 seconds in each case. A solvent ratio of 1:10 for fresh samples and a ratio of 1:20 for dried samples were used, taking into consideration the moisture content of the samples. After the microwave treatment, the mixtures were filtered and centrifuged at 13000 rpm at 14 °C temperature for 20 min using a Hettich Zentrifugen MICRO 22R centrifuge.

Analytical methods

Determination of total phenolic contents (TPC) by Folin-Ciocalteu method: The Folin-Ciocalteu spectrophotometric method by Singleton and Rossi [8], at 760 nm is an electron transfer based assay and shows the reducing capacity, which is expressed as phenolic content. Gallic acid (GA) was used to prepare the standard curve. The results were expressed as μ M GA/g of dry matter (DM), and fresh matter.

Determination of antioxidant capacities by FRAP (Ferric Reducing Antioxidant Power) method: Measurement of ferric reducing antioxidant power of the peel extracts was carried out based on the procedure of Benzie and Strain [9], at 593 nm. Ascorbic acid (AA) was used as a standard to prepare the calibration solutions. Results were expressed as μ MAA/g DM, and fresh matter.

Determination of carotenoid content:

The carotenoid content of all extracts was determined spectrophotometrically (Yang et al., 1998) [10] (in addition to chlorophylls), expressed in $\mu g/g$ for both fresh and dried samples.

Results and discussion

Figure 1 shows that the extraction with 20 % alcohol resulted in the leaching of more polyphenolic components due to the variable solubility of polyphenols (Figure 1). Compared to the control, the amount of leached polyphenols was the highest at 100 W microwave power. In conclusion, this leaching method was found sufficient for the exploration.

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Figure 1. Polyphenol content of fresh and dried carrot peel samples using distilled aqueous and alcoholic solvents and different power levels

Control distilled water Distilled wa	ter Control alcohol	Alcoholic extract
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The results showed that the optimal valuable substance extraction was obtained at 1:10 peelsolvent ratio at 100 W microwave power for fresh carrot peels, and 1:20 peel-solvent ratio at 800 W microwave power for dried carrot peels.

The same conclusion was drawn for antioxidant capacity (FRAP) (Figure 2).



Figure 2. Antioxidant capacity (FRAP) of fresh and dried carrot peel samples using distilled aqueous and alcoholic solvents and different power levels

The two adjacent graphs (Figure 3) show the carotenoid content of alcoholic extract of fresh respectively dried carrot peel samples at different power levels. The conclusion was that the carotenoid content of both samples increased with performance enhancement. Furthermore, the carotenoid content of the dried samples was over twice as many as the carotenoid content of the fresh samples.

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Figure 3. Carotenoid content of fresh and dried carrot peel samples in alcohol extraction using a solvent ratio of 1:10 for fresh samples and 1:20 for dried samples

Conclusion

Based on the results obtained, it is safe to assume that the extraction procedure with 20 % ethyl alcohol proved to be the most efficient for the extraction of the tested components in the case of both fresh and dried carrot peel samples. Considering the whole experiment, the extraction at 100 W proved to be the most efficient for fresh carrot peels

Based on the presented preliminary experiments, it can be concluded that microwave-assisted extraction is suitable for the leaching of valuable endogenous components from carrot peel available in large amounts as food industry waste, and it is worthwhile to address the further utilization of this by-product.

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THE APPLICATION OF PHENYL COLUMN AS A NEW CHALLENGE IN ANISOTROPIC LIPOPHILICITY DETERMINATION OF 17-PICOLYL AND 17-PICOLINYLIDENE ANDROSTANE DERIVATIVES BY RP-UHPLC METHOD

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Abstract

Androstane derivatives are highly-potent compounds with anticancer activity. The lipophilicity is one of the most important physicochemical parameters of biologically active compounds. Experimental determination of lipophilicity takes into account numerous approaches among which is chromatography as one of the most reliable analytical methods. Usually, the chromatographic determination of lipophilicity of biologically active compounds is based on application of C₁₈ or C₈ columns. In the case of 17-picolyl and 17-picolinylidene androstane derivatives C_{18} column has been applied earlier. However, this study presents the challenge in the lipophilicity determination by using phenyl column in reversed-phase ultra-high performance liquid chromatography (RP-UHPLC) system. The method with the phenyl column is specific due to the presence of π - π interactions between the phenyl column and π -electron containing 17-picolyl and 17-picolinylidene androstane derivatives. The mobile phases used were three types of mixtures including methanol/water mixture, acetonitrile/water mixture and methanol/acetonitrile/water mixture. The fraction of water in mobile phases varied from 20 to 30 v/v. The results of the chromatographic analysis showed a good separation of the analyzed androstane derivatives and provided chromatographic parameters (capacity factor $-\log k$) well correlated with *in silico* lipophilicity descriptors (partition coefficient $-\log P$). Therefore, the logk values, obtained by using phenyl column, of the analyzed androstane derivatives can be considered to be their lipophilicity measures or chromatographic (anisotropic) lipophilicity measures.

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IMPROVING METHANE PRODUCTION FROM LIGNOCELLULOSIC BIOMASS PRE-TREATED WITH ANAEROBIC FUNGI

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Abstract

Two anaerobic fungi (AF) strains, isolated from faeces of an elephant and of a sheep, were assessed for their ability to degrade lignocellulosic biomass. The effects on biogas production of anaerobic fungi from both animal species were determined in two step batch experiments. The hydrolysis process during the AF incubation led to an initial increase of biogas production, an accelerated degradation of dry matter and an increased concentration of volatile fatty acids. Thus, a separate hydrolytic pre-treatment phase with anaerobic fungi, represents a feasible strategy to improve biogas production from lignocellulosic substrates.

Introduction

Degradation of lignocellulose-rich material into biogas is an attractive strategy to face growing energy demands and moderate greenhouse gas emissions from the exploitation of fossil energy resources. Lignocellulosic residues (e.g. crop residues, green waste, mill waste) are highly frequent (1), they are easily accessible, cheap and do not require additional land to grow on in this way do not trigger "food or fuel" conflicts. This biomass is composed of interwoven cellulose and hemicellulose, coated by recalcitrant lignin (2). This is the explanation why bacteria and archaea in the biogas reactor are not efficient in disintegration of the lignin, leaving a considerable portion of the more easily convertible sugars untouched. Current strategies to release this carbon rely on expensive enzyme cocktails and physicochemical pre-treatment, producing inhibitory compounds that hinder subsequent microbial bioproduction. Microbial pre-treatment utilizing the fibre degrading potentials of aerobic fungi may be a much cheaper alternative but there are some drawbacks e.g. loss of carbohydrates by respiration and biomass build-up and the requirement of long pre-treatment periods (3). Anaerobic fungi (AF) from the phylum Neocallimastigomycota are natural inhabitants of the digestive tract of herbivorous animals (4), which decompose a big share of the ingested forage. The AF attach to the plant material and crack the fibres mechanically by growth and expansion of their rhizoids or bulbous holdfasts (5). In addition, AF possess cellulosomes which contain a multitude of lignocellulolytic enzymes. These fungi are an appealing solution as they hydrolyze crude, untreated biomass at ambient conditions into sugars that can be converted into value-added products by partner organisms.

Experimental

The objective of this study was the application of two newly isolated strains to the hydrolysis phase in order to improve hydrolysis of lignocellulosic biomass. The applied isolates were obtained from animals living on a high fibre diet, namely sheep (*Ovis aries*) (Fig.1.) and Asian elephant (*Elephas maximus*). The effects on biogas production of anaerobic fungi from both

animal species were assessed in two step batch experiments, comprised by a hydrolytic/acidogenic stage, followed by a methane production stage. Checking the enzyme activity in hydrolytic stage, beta-glucosidase activity was measured by p-nitrophenyl- β -D-glucopyranoside, for the endoglucanase concentration DNSA-method was used (3,5-dinitrosalicylic acid). The produced organic acids were measured by HPLC. Additionally, gas composition was analysed by GC during the methane production stage.





Results and discussion

In this study, treatment with anaerobic fungi cultures increased the total biomethane yield during the experimental period of 20 days. After the pretreatment the medium was taken apart into liquid and solid phases, which were treated separately. Columns show the difference between the pretreated and non-treated data (Fig. 2.).



Figure 2. Biomethane yield after the anaerobic fungi pre-treatment.

Pretreatment with anaerobic fungi significantly improved the degradability of straw. This is evidenced by the fact that about 2.5 times more biomethane was produced from the elephant

solid and sheep solid samples during the experiment than from the untreated straw (Fig. 2.). The results for the elephant fluid and sheep fluid samples correlated well with the organic acid concentrations measured by HPLC (Fig.4.) and the high endoglucanase and beta-glucosidase results measured during the tests (Fig. 3). From these it can be concluded that the anaerobic fungi degraded the substrate efficiently during the 15-day long treatment. As a result of the pre-treatment, acetic acid, lactic acid, glucose and cellobiose were produced in the highest amounts. These products could be used by methane-producing Archea producing methane during biogas fermentation. The efficiency of biogas fermentation was well characterized by the amount of biomethane produced as well as the concentrations of organic acid measured by HPLC (Fig.4.), which show that methanogens with otherwise slow metabolism were able to use the products of anaerobic fungi from the solution, so inhibition did not occur.



Figure 3. Enzyme activities during wheat straw fermentation using sheep-AF isolate.



Figure 4. Organic acid concentration at the end of the biogas fermentation using anaerobic fungi from sheep.

Conclusion

Anaerobic fungi isolates from sheep and elephant are excellent candidates for the conversion of agricultural waste products to biofuels. The two tested isolates efficiently pretreated the hard-to-degrade straw substrate to produce significant amounts of acetic acid, lactic acid, glucose, and cellobiose. The kinetics of the degradation were optimal for the slow metabolism of methanogenic microbes, which were thus able to efficiently utilize the aforementioned by-products and make biomethane from them.

Based on these results AF isolates were effective in enhancing cellulose degradation and successfully increased biogas production.

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ANALYSIS OF TRACE AMOUNTS OF ESTROGENIC COMPOUNDS IN HIGH VOLUME WATER SAMPLES BY SPE-UPLC-MS/MS

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Abstract

In this work, a solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and optimized for the simultaneous analysis of six different estrogenic compounds in water. The developed method is suitable for the determination of the selected compounds at the low ng/L level. Real environmental water samples will be analyzed using this method.

Introduction

Today's increasing urbanization and rapidly growing industrial and agricultural activity release more and more contaminants into the environment. Freshwater pollutants of anthropogenic origin are a global health concern now. A diverse group of these emerging contaminants consists of endocrine disrupting compounds (EDCs). EDCs disturb the endocrine system of aquatic and terrestrial organisms, causing decreased fecundity, altered mating behaviour, developmental disorders, and thyroid dysfunction [1], [2]. Epidemiological studies suggest associations between chronic human exposure to EDCs and reproductive dysfunctions or civilization diseases, as well. Thus, there is a growing interest towards quantitative information about endocrine disruptors in freshwaters.

Experimental

The aim of this work was to develop a solid phase extraction (SPE) and liquid chromatographytandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of six different EDCs, including three estrogens (17-D-estradiol, 17-D-ethinylestradiol, estrone) and three industrial chemicals (bisphenol A, bisphenol F, bisphenol S) in water. Ultrapure water was acidified to pH=3 and spiked with the target analytes at 100 ng/L. Solid phase extraction (SPE) was carried out by an automata SPE instrument (Dionex AutoTrace 280, ThermoFisher Scientific). SPE conditions were optimized by testing different types of SPE cartridges and eluents. For signal enhancement, chemical derivatization was carried out. Target analytes were incubated with dansyl chloride at 65 °C for 10 minutes under alkaline conditions. Instrumental analysis of the target compounds was performed by a Waters ACQUITY UPLC H-Class System, coupled with an Xevo TQ-S micro triple quadrupole mass spectrometer equipped with Electrospray Ionization source operated in negative ion mode detecting native compounds and in positive ion mode detecting dansylated derivates. The chromatographic separation was performed on Waters Aquity UPLC BEH C18 column. The mobile phase consisted of water and methanol, containing 0,1% formic acid when analyzing dansylated derivates. The quantitative analysis of the target compounds was performed in multiple reaction monitoring (MRM) mode.

Results and discussion

Limits of detection (LOD) for the selected native EDCs varied from 0,05 to 7,5 ng/L detected in negative ion mode. Chemical derivatization of the target compounds with dansyl chloride improved the sensitivity of the method. As the electrospray ionization technique is generally more effective for ionization of polar or ionic substances than non-polar compounds, chemical derivatization of native steroid estrogens with low polarity enhances their poor ionization efficiency. The reaction of steroid estrogens with dansyl chloride produces derivates containing easily ionizable basic N-atoms, resulting in enhanced sensitivity in ESI positive ion mode [4], [5]. The signal intensities of dansyl derivates were remarkably higher compared to native compounds. The LOD values were by an order of magnitude lower in positive ion mode after derivatization.

Conclusion

The developed method is suitable for the simultaneous determination of trace amounts of the selected six EDCs in water. Chemical derivatization with dansyl chloride significantly improved the sensitivity of the method. The LOD values of the target analytes were an order of magnitude lower in positive ion mode after derivatization compared to the related native compounds analyzed in negative ion mode. The developed method will be applied to real environmental samples.

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HIGH ENERGY IONIZING RADIATION INDUCED DEGRADATION OF β-BLOCKERS IN AQUEOUS SOLUTIONS

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Abstract

Degradation reactions of two beta-blockers, atenolol and propranolol were studied using high energy ionizing irradiation interpreting the outcome of the ongoing radical reactions on the degradation efficiency, oxidation and mineralization processes and toxicity. Under appropriate conditions both hydroxyl radical ('OH) and hydrated electron (e_{aq}) take part in degradation reactions. Propranolol showed higher reactivity under both oxidative end reductive conditions than atenolol. Thus it is not surprising that the oxidation and mineralization reactions take place more rapidly due to its condensed ring having higher electron density on the aromatic ring in propranolol. During removal of propranolol toxic products, hydroxylated naphthalene derivatives form. Using appropriate doses the starting molecules can be degraded and the toxic character of the end-products can be eliminated.

Introduction

Beta-blockers are applied for treatment of cardiovascular diseases. These molecules contain an aromatic ring and an oxypropanolamine side chain. Both beta-blockers and their metabolites can be detected in wastewater effluents and surface waters in ng- μ g dm⁻³ concentration level [1-2]. Their elimination has been already investigated by a wide range of Advanced Oxidation Processes [3-5].

In this study the removal efficiency and degradation mechanism of two frequently used betablockers, atenolol and propranolol was investigated in details supplemented by monitoring the change of the organic content and the toxicity.

Experimental

Atenolol and propranolol dihydrochloride were obtained from Sigma-Aldrich. *Tert*-butanol was produced by Molar Chemicals, it was used in order to remove the hydroxyl radicals from the system when the hydrated electron reactions were studied. In pulse radiolysis experiments buffer solution was used from K_2HPO_4 and KH_2PO_4 . Purified water with a conductivity of 0.055 μ S cm⁻¹ and total organic content of < 2 ppb was provided by an Adrona B30 system. The solutions were air equilibrated or bubbled with N_2 or N_2O continuously during the measurements depending on the reaction investigated.

Pulse radiolysis experiments were performed using 4 MeV accelerated electrons with electron pulse length of 800 ns and an optical system with 1 cm path length cell [6-7]. The measurements were performed in 0.1 mmol dm⁻³ atenolol and propranolol solutions at pH 7. The formation and decay of the transient intermediates were monitored in the reactions of atenolol and propranolol under oxidative and reductive conditions. The rate constants for these reactions were measured by transient kinetic measurements.

The γ -radiolysis experiments were carried out in a panoramic type 60 Co γ -chamber with a dose rate of 10 kGy h^{-1} at room temperature. The reactions of various reactive intermediates from water radiolysis were studied under specific reaction conditions. In N₂ saturated solutions all

the three radical intermediates of water radiolysis, hydroxyl radical, hydrated electron and hydrogen atom are reacting agents. In the presence of *tert*-butanol e_{aq}^- is the main reaction partner, but there is a small contribution from the H[•] reactions. In the presence of O₂, i.e., in air equilibrated solutions e_{aq}^- and H[•] transform to the low reactivity O₂[•]/HO₂[•] pair (pK_a = 4.8). Hydroxyl radical reactions are generally investigated in N₂O saturated solutions [8-9].

The end-products were featured by ultraviolet–visible (UV–Vis) measurements, chemical oxygen demand (COD), total organic carbon (TOC) and toxicity. The un-irradiated solutions and solutions irradiated under different conditions were measured by a JASCO 550 UV-Vis spectrophotometer in 1 cm cell. In COD and TOC measurements a Behrotest TRS 200 COD system and a Shimadzu TOC-LCSH/CSN equipment was applied. In acute toxicity tests *Vibrio fischeri* luminescent bacteria was used as test organism detecting the changes in the luminescence of the bacteria, caused by the chemicals tested.

Results and discussion

Pulse radiolysis

In N₂O saturated solutions (c = 0.1 mmol dm⁻³) the reactions of 'OH with atenolol and propranolol were studied, respectively (Fig. 1, A and B). In the case of atenolol a wide, double band with maxima at 310 and 325 nm was observed. For propranolol a more narrow, sharp peak appeared at $\lambda_{max} \approx 325$ nm with a shoulder at the longer wavelength side and a wide and a flat band at 380 nm. Similar transient absorption spectra with two bands at 320 and 370 nm were measured in aqueous naphthalene solution [10-11]. The transient absorbances in Fig. 1 decayed on the several times 100 µs timescale, during the decay no major changes in the shapes of the spectra were observed. In the reaction between aromatic molecules and 'OH several hydroxycyclohexadienyl isomers form, these intermediates exhibit light absorption in the of 300-400 nm range.



Figure 1. Absorption spectra of transient intermediates of 'OH reactions in N_2O saturated, 0.1 mmol dm⁻³ atenolol (A) and propranolol (B) solutions, respectively. Insets: concentration dependence of pseudo-first-order rate constant of product build-up at 325 nm for both molecules.

The e_{aq} reactions were also studied for both beta-blockers (not shown). For atenolol very weak absorbances were measured in the 300-350 nm range, whereas a strong absorption spectrum can be observed at 325 nm and 380 nm appeared in the e_{aq} + propranolol reaction, similar to that obtained in the 'OH + propranolol reaction (Fig. 1 B). Protonation of electron adduct is expected to give similar radical intermediate that also forms in H-atom addition to the naphthalene unit.

Kinetic measurements were carried out to determine the rate constant of 'OH and e_{aq} reactions. In 'OH reactions the second-order rate constants were found to be 4.80×10^9 and $7.55 \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ for atenolol and propranolol, respectively (Fig. 1 Insets). This difference can be interpreted by the chemical structure of the two molecules: propranolol possesses a condensed ring with higher electron density and more vulnerable sites than atenolol having a simple aromatic ring. In e_{aq}^{-} reactions the rate constants of $5.8 \times 10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ and $8.6 \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ were measured for atenolol and propranolol, respectively.

UV-Vis measurements

The effect of different reaction conditions on the degradation efficiency was followed up by UV-Vis measurements (Figures 2 and 3). In the UV spectra there are two absorption bands for atenolol: one at 225 nm, and a second one (the typical wide aromatic band) is between 250 and 290 nm. In the case of propranolol both bands are wider, they are at 221 nm and between 250 and 320 nm. When 'OH participated as a reacting agent in the degradation (in air, N₂ and N₂O saturated solutions) slight shifts of the absorbance maxima were observed to the longer wavelengths. This shift can imply the presence of forming hydroxylated products in irradiated solutions having absorbance maxima at longer wavelengths than the initial molecule. In such solutions increase in the baseline was also marked due to the light scattering in the presence of some badly soluble products. When 'OH is the main initiating radical about 1 kGy dose is sufficient to degrade practically all the initial atenolol or propranolol molecules.

In N₂ saturated solutions containing *tert*-butanol (reaction partner e_{aq}) different changes can be observed. The hydrated electrons practically do not degrade atenolol. By contrast, abatement and shift of the band between 250 and 320 nm to lower wavelengths can be observed in the case of propranolol. This degradation is almost as intensive as in the case of 'OH reaction.



Figure 2. UV-Vis absorption spectra in 0.1 mmol dm⁻³ irradiated atenolol solutions under different conditions in the 0-1 kGy dose range: 'OH and O_2^{-}/HO_2^{-} (A), 'OH and e_{aq}^{-} (B), 'OH (C) and e_{aq}^{-} (D).



Figure 3. UV-Vis absorption spectra in 0.1 mmol dm⁻³ irradiated propranolol solutions under different conditions in the 0-1 kGy dose range: 'OH and O_2^{-}/HO_2^{-} (A), 'OH and e_{aq}^{-} (B), 'OH (C) and e_{aq}^{-} (D).

Oxidation and mineralization

In the presence of O_2 the solutes gradually transform to more and more oxidized molecules (oxidation), finally they end up as inorganic species, H₂O, CO₂, etc. (mineralization). The oxidation and mineralization are often followed up by the changes in the dissolved O_2 and organic carbon atom contents of the solutions using chemical oxygen demand (COD, mg O_2 dm⁻³) and total organic carbon (TOC, mg C dm⁻³) measurements (Fig. 4). Both the COD and TOC values decrease with the increasing dose. The initial rates of oxidation were 8 and 13 mg dm⁻³ kGy⁻¹ in atenolol and propranolol solutions. At the early stage of the degradation mainly the initial compound and its slightly transformed products are present in the solution which oxidize more readily than the small molecular fragments (aldehydes, carboxylic acids, etc.) dominating the organic content present at higher doses [12]. At 10 kGy, 58.8 and 65.4% COD decline was observed in aerated solutions for atenolol and propranolol, respectively.

The TOC values decrease almost linearly in the dose range investigated. The mineralization rates were 0.9 and 1.4 mg C dm⁻³ kGy⁻¹ for atenolol and propranolol, respectively, the processes were more effective in the case of propranolol.



Figure 4. The dose dependence of the oxidation and mineralization in aerated, 0.1 mmol dm⁻³ atenolol and propranolol solutions.

Toxicity assays

Vibrio fischeri bioluminescence toxicity assays were applied to follow up the change of toxicity in irradiated solutions equilibrated with air. The initial concentration was 0.1 mmol dm^{-3} similar

to the former measurements. Neither atenolol, nor propranolol did show toxic effect in the initial concentration in agreement with their published high EC_{50} values, 5 and 0.3 mmol dm⁻³ for *Vibrio fischeri* [13]. In the case of atenolol luminescence inhibition of the degradation products was below 10% in the entire dose range. The products of propranolol were more toxic than the products of atenolol. In propranolol solutions irradiated with 0.4 kGy dose ~80% inhibition was observed. At higher doses the toxicity of products decreased below 15%.

Conclusion

Both atenolol and propranolol degrade *via* radiolytic processes in which 'OH may predominantly react with the aromatic ring in 0.1 mmol dm⁻³ aqueous solutions. Due to the presence of the condensed ring propranolol has higher reactivity towards both 'OH and e_{aq}^{-} than atenolol, thus the oxidation and mineralization reactions proceed also with higher rates in the case of propranolol. In the case of propranolol the end-products with high toxicity may also form at low doses as highly toxic hydroxylated naphthalene derivatives may evolve in 'OH reactions. Removal of atenolol and propranolol, as well as abolishment of the toxicity can be achieved using appropriate doses.

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NATURAL CHIRAL CATALYSTS ON SOLID SURFACES USED IN MICHAEL ADDITION TO MALEIMIDES

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Abstract

As the most important molecules in living organisms are chiral, the selective production of the optical isomers of substances designed to interact with them, such as drugs, is of paramount importance. In addition, from an environmental point of view, it is also desirable to carry out the asymmetric syntheses as efficiently as possible, thus minimising waste production. Asymmetric catalytic syntheses are one of the best methods for producing enantiomerically pure compounds, whereby the heterogenization of the chiral catalysts can allow their reuse. A simple method to heterogenize chiral catalysts is the adsorption of optically pure material on a solid surface. Some examples of the use of heterogeneous chiral catalysts that can be readily prepared by adsorption are known, however, solids of this type have not yet been used in reactions of maleimides.

Based on these, we set out to explore the combined catalytic effect of natural amino acids and various inorganic oxides in Michael additions between maleimides and aldehydes. The investigation of a variety of starting materials provides an opportunity to study the activity, stereoselectivity and the applicability of these simple chiral catalysts. Our studies have shown that while natural amino acids have only negligible catalytic activity, adsorption on different oxide surfaces (alumina, bentonite or laponite) gave succinimide derivatives with excellent conversions and enantioselectivities. In conclusion, our research has led to the development of a novel chiral heterogeneous catalytic system that can be used to produce valuable optically pure intermediates for use in the pharmaceutical industry.

Introduction

Nowadays, asymmetric organocatalysis is one of the most dynamically developing area of the synthetic chemistry. Inexpensive, readily available natural chiral compounds, such as alkaloids, tartaric acid, and amino acids, are often used to prepare common asymmetric organocatalysts [1]. A major disadvantage of catalysts soluble in the reaction media is that they are difficult to recover, thus the possibility of reusability is lost in many cases.

So-called atom-economic and sustainable processes are also becoming important in the fine chemical industry. In the field of organocatalysis, reactions with asymmetric heterogeneous catalysts are of increasing importance, in which the solid source of chirality can be mechanically separated from the reaction mixture, thus allowing its reusability [2-4]. One of the simplest ways of heterogenization is to attach the homogeneous chiral catalyst to a support that is not soluble in the reaction mixture. Natural amino acids are inexpensive, readily available chiral organocatalysts, but only few catalytic systems are known in which they have been immobilized by adsorption or hydrogen bonding despite this may improve their activity and stereoselectivity in asymmetric reactions [5].

The organocatalysts have various applications in asymmetric C-C coupling reactions, such as the synthetically important Michael addition reactions. The diversity of the starting materials allows the synthesis of a wide range of products of practical interest. Among the Michael acceptors used, maleimide derivatives are outstanding because the succinimides formed can be used as valuable intermediates in the pharmaceutical industry [6,7]. There are many succinimide derivatives in the literature that have been applied for the treatment of cancer, epilepsy, the HIV virus infection and degenerative diseases [8,9].

One of the simplest ways to bind the chiral material to the surface of the support is by simple adsorption. An amino acid-hydrotalcite hybrid catalyst has recently been prepared by high-speed ball milling, which afforded excellent results in the Michael addition of maleimides and aldehydes [10]. A major advantage of heterogeneous catalysts created by second-order interactions is that they do not need to be attached to the insoluble support in a preliminary step, as they self-assemble *in situ*. We aimed to use natural amino acids adsorbed on inorganic oxide surfaces as chiral catalysts in Michael additions between maleimides and aldehydes. Our plan was to study the effect of the structure of the α -amino acid and the influence of the applied inorganic oxide on the outcome of the C-C coupling in a selected test reaction, *i.e.* the addition of isobutyraldehyde to *N*-benzylmaleimide, which we have previously investigated using bifunctional homogeneous organocatalysts [11]. Our goal is to develop an environmentally friendly catalytic system in which a new, efficient and reusable catalyst is applied.

Experimental

Natural amino acids were purchased from Sigma-Aldrich and used as received. Isobutyraldehyde and a few *N*-substituted maleimides were commercial products and were used without purification. Solvents, reagents and inorganic additives of analytical grades were used in all reactions. To prepare new maleimides we purchased analytical grade primary amines and maleic anhydride. Maleimides were synthesized using sodium acetate and acetic anhydride in one day at 70°C. The crude products were purified by flash chromatography. The hybrid catalyst was prepared *in situ* in the reaction mixture.

Catalytic Michael additions were performed in vials with magnetic stirring. The chiral catalyst and the inorganic oxide were suspended in the given solvent, followed by the addition of a maleimide derivative and four equivalents of isobutyraldehyde. The mixture was stirred at the indicated temperature. After the given time, the products were separated by centrifugation and analysed by GC-MSD and GC-FID using chiral capillary columns. Larger scale experiments were also carried out and the resulted products were purified by flash chromatography for determination of the isolated yields. The pure compounds were characterized by ¹H- and ¹³C-NMR spectroscopy. The chiral hybrid materials were investigated by FT-IR and Raman spectroscopy, XRD measurements and scanning electron microscopy.

Results and discussion

Due to the prominent importance of succinimides, our primary goal was to develop a sustainable catalytic system for the C-C coupling reaction of maleimides and aldehydes in an enantioselective manner. Our research group has already investigated a similar catalytic system in other Michael additions [5], which provided a good starting point for the design of our catalytic system. L-phenylalanine was selected as chirality source on the basis of literature results and applied in our test reaction by *in situ* adsorption on different oxide supports (Scheme 1.) [7,10].





In the first step, we investigated the effect of inorganic oxide quality in the Michael addition of *N*-benzylmaleimide and isobutyraldehyde using L-Phe natural amino acid (Figure 1). It can be seen on the figure that the reaction proceeds with minimal transformation without solid support. Using acidic oxides the conversion of succinimide is low, similar with the reaction carried out without additive. Al₂O₃ with a slightly basic surface and the cation-exchanger clays Laponite (Lap-RD) or Bentonite (Ben-H) with layered structures provided excellent conversions and high enantioselectivities.



Figure 1. Testing the quality of inorganic oxide supports.

In our further studies Bentonite was chosen from these inorganic supports, because it is one of the cheapest, easily obtainable clay mineral. In the next step, we investigated the effect of the amount of this oxide, which is shown in Figure 2. Very small amounts of the oxide support are sufficient to form close to optically pure succinimide by complete transformation of the maleimide. It can also be seen that the amount of oxide has no effect on the enantioselectivity but it has an effect on the reaction rate.



Figure 2. Effect of the amount of bentonite in the test reaction.

The effect of amino acid structure was also investigated (Scheme 2.). The results show that both the carboxylic acid group and the amino group play a key role in the catalysis. When other

open-chain primary amino acids were used, only moderate conversion values and low enantioselectivities were obtained, so that in comparison with phenylalanine, it appears that carbon chain quality plays a significant role in steric hindrance. The amino acid with a ring structure (L-Pro) is not properly involved in the catalysis.



Scheme 2. Testing different amino acids on bentonite in the test reaction.

The best performing catalyst, natural L-Phe on bentonite, was used to optimize the reaction conditions. The effects of temperature, reaction time, solvent and reactant quantity and quality were studied to achieve full conversion of the maleimides and isolation of the corresponding products with high enantioselectivity. Several promising maleimide derivatives, which are not commercially available, were identified as potential candidates for reaction and were prepared and purified. The results in Scheme 3. show that maleimides containing aliphatic groups at the *N*-position also gave good results, but did not reach the values obtained in the presence of the aromatic system. Thus, we could extend of the applicability of the reaction in Michael additions with isobutyraldehyde in the catalytic system described above, which opened a favorable route to the preparation of optically pure succinimides with diverse structures.



Scheme 3. Using L-Phe and BenH hybrid heterogenous catalyt in the test reaction.

Accordingly, we have succeeded in developing heterogeneous catalysts using inexpensive natural amino acids and readily available inorganic oxides that retain their activity after repeated use, rivalling the results of catalysts used in the homogeneous system. This represents a major step forward towards environmentally friendly heterogeneous catalytic methods as opposed to the use of expensive chiral organocatalysts.

Conclusion

In our research, we have developed a catalytic system that has the potential to produce valuable optically pure intermediates for the pharmaceutical industry. We have succeeded in developing low-cost, solid, reusable chiral catalysts with natural amino acids and inorganic oxides, which can be used to achieve similar or better results than the soluble chiral catalysts used to date. The

applicability of the organic-inorganic hybrid catalysts were investigated in several asymmetric conjugate additions to various maleimides. With this organocatalyst, high conversions and enantioselectivities can be achieved in Michael additions of aldehydes, thus provided and environmentally benign method for the preparation of optically pure succinimides. This represents a major step forward in the application of heterogeneous catalysis in the pharmaceutical industry for the environmentally friendly and sustainable production of optically pure intermediates.

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THE FORMATION KINETIC OF MECHANOCHEMICAL SYNTHESIZED PEROVSKITES

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Abstract

In this study, we aimed to achieve mechanochemical perovskite synthesis and to quantify the energy used during milling (E_b - impact energy and E_{cum} - cumulative energy) and to describe the relationship between them. For mechanochemical treatment a Fritsch Pulverisette-6 type planetary ball mill was used. As a model compound the widely used barium-titanate (BaTiO₃) was chosen, which was produced by the reaction of barium-oxide (BaO) and titanate-dioxide (TiO₂). The aim was to track the formation of BaTiO₃ and to determine the minimum milling energies required for its production. Three important parameters were considered for the calculation of energy values: the material of the milling vials and balls, the number of balls and the speed of rotation. The transformation was tracked by X-ray diffraction (XRD) measurement, and the applied energy was determined using the Burgio-Rojac energy model. Our goal was to draw conclusions that can be used to predetermine optimal milling parameters in the production of other perovskite structured materials. The hypothesis was verified by the mechanochemical synthesis of zinc-titanate (ZnTiO₃) which was produced by the reaction of zinc-oxide (ZnO) and TiO₂.

Introduction

Looking at the main three-component crystal structures, it found that of the thousands of complex structures, there are only a dozen ceramics that are significant in use. Among these, the A_2BX_4 spinel and ABX_3 perovskite structures stand out, and perovskite is the only structure, the chemical modification of which results in an extremely wide range of phases with completely different properties. Due to its unique electrical properties, the family of chemical compounds with a perovskite-type structure includes a wide range of electrotechnical materials: semiconductor dielectrics, superionic conductors, combined with ionic and electron conductivity for high-temperature superconductors. [1,2]

Mechanical activations and chemical reactions in planetary ball mills have long been known, but there are still challenges. There are many factors that influence the success of mechanochemical reactions: the material of the milling vial and balls; the rotational speed; the milling time; the number of balls and the filling ratio of the balls and reactants; the atmosphere and temperature in the vial, the physical and chemical properties of the reactants etc. These parameters are not independent of each other and play an important role in achieving optimal treatment, which results the best available yield. [3] For any combination of the factors above, the milling time in the given system must be determined separately. The intensity of milling energy affects the increase in the particle size of crystalline materials, and as the temperature changes, compounds of different compositions may be formed. [4] It should be considered that too long milling process can result in undesirable products, while insufficient treatment does not allow for proper conversion of starting materials.

Experimental

For mechanochemical treatment a Fritsch Pulverisette-6 type planetary ball mill was used. Each milling drum has a volume of 80 mL, the milling balls were 10 mm in diameter and 2.00 g BaO and 1.04 g TiO₂ were measured in the vial in each case. Based on the mass of the balls and the reactants weighed, the minimum and maximum ball-to-powder ratios can be determined in each milling drum. This number varied between 5.5-1 and 61.5-1. We were able to control this by milling vials made of different materials (silicon nitride Si_3N_4 , hardened stainless steel FeNiCr, hard metal tungsten-carbide WC), we were able to change the milling energy within a wide spectrum. The transformation of starting materials was followed by XRD.

The Burgio-Rojac equation (1) can be used to determine two energy values: the E_b (1), which represents the total energy available during an impact event of a milling ball, and E_{cum} (2), which means the energy transferred to 1 gram of the powder during whole milling:

$$E_b = \frac{1}{2}\varphi_b K\left(\rho_b \frac{\pi d_b^3}{6}\right) \omega_p^2 \left[\left(\frac{\omega_v}{\omega_d}\right)^2 \left(\frac{d_v - d_b}{2}\right)^2 \left(1 - 2\frac{\omega_v}{\omega_d}\right) - 2r_p \left(\frac{\omega_v}{\omega_d}\right) \left(\frac{d_v - d_b}{2}\right) - \left(\frac{\omega_v}{\omega_d}\right)^2 \left(\frac{d_v - d_b}{2}\right)^2\right] (1)$$

where *K* is the geometric constant of the mill, φ_b is the obstruction factor, ρ_b is the density of the milling balls, d_b is the diameter of the balls, d_v is the diameter of the milling vial, ω_p and ω_v is the rotational speed of the disc and the crucible and r_p is the distance between the rotational axes of the disc and the crucible.

$$E_{cum} = \frac{E_b \times f \times t}{m_p} \tag{2}$$

where f is the frequency of impacts, t is the milling time and m_p is the mass of the measured sample. [5]

In addition to $BaTiO_3$, we also produced $ZnTiO_3$ by mechanochemically. In these experiments, according to the stoichiometry of the reaction, 1.50 g of ZnO and 1.47 g of TiO₂ were measured in the vial in each case. This was necessary because the total weight had to be kept at around 3 grams, so that the ball-to-powder ratios previously used for $BaTiO_3$ could be interpreted in this case as well.

Results and discussion

Based on the Burgio-Rojac equation, E_b , E_{cum} values and the frequency of impact of the balls were calculated for each sample. By depicting these data, the so-called energy map of a milling series can be prepared. **Fig.1.** illustrates the relationship between E_b and the material of the applied vial and the frequency of impact. In case of Si₃N₄, only 300, 400 and 500 rpm, and for FeNiCr and WC vial data on samples milled at 200 rpm are also indicated. This can be explained by the fact that in samples milled at this value (Si₃N₄), the conversion of the starting materials was not occured at all, it started only at 300 rpm with the use of 25 milling balls.



Figure 1: E_b points defined by the Burgio-Rojac equationas a function of the milling vials (left) and the frequency of impacts (right).

Fig.1. shows that in the case of higher density milling vials, increasing the speed has a much more significant impact on the dynamics of the growth of E_{b} . With the tungsten-carbide vial a much wider energy interval can be covered, but the minimum speed will determine its resolution, however in the case of the lower density Si_3N_4 vial this much more precisely controllable. [6]

During milling, an hourly sample was taken of, which was measured immediately with XRD. **Fig.2.** shows X-ray diffractograms of samples milled in different vials with the same setting (400 rpm, 20 balls). As expected, the formation of Ba/ZnTiO₃ differs significantly in the three vials.



Figure 2: XRD of samples milled with the same parameters (400 rpm, 20 balls). The 0-hour sample is made of the BaO/ZnO-TiO₂ starting materials mixture.

The E_b are almost doubling in the case of milling vials treated with the same parameters but having different material. This is reflected in the XRD recordings, where a fundamental difference can be found that the production of BaTiO₃ is already sufficient for a lower E_b value, resulting in 35.5 J/hit. Typical reflections appear already during treatment in the FeNiCr vial after 1 hour. In the case of ZnTiO₃, the transformation of the starting materials in the Si₃N₄ vial does not take place at this E_b value, and the reflections characteristic of crystalline $ZnTiO_3$ appear in the FeNiCr drum only after 2 hours.

To better represent the measured results, diffractograms were read and used to track the formation of reactants at the intensity of most intense BaTiO₃ peaks (2θ 31.4°), i.e. based on the fact that the intensity of this peak shows the increasing appearance of the product in the vial. The same was done for ZnTiO₃ based on reflection of 20 36.3°. The results are presented in **Fig.3**.



Figure 3: XRD measurement-based formations of BaTiO₃ and ZnTiO₃ of the samples in different milling vial.

Good correlation between the performed E_b and the conversion rate is observed in the case of BaTiO₃ and ZnTiO₃ also. E_b which is necessary to produce the perovskite structure, begins at a nearly similar value, however, BaTiO₃ is already formed at ball-impact energy of 0.12 J/hit, while the same is only done at 0.25 J/hit in the case of ZnTiO₃. By this way, it can be stated that the thresholds E_b to produce the BaTiO₃ perovskite is correspond to the above values.

Conclusion

By increasing the milling time, we could increase the E_{cum} , which is able to correct the low E_b to a certain extent, but it should be noted that in this case the perovskite crystal structure may be damaged. For both perovskites, the formation of the structure can be achieved mechanochemically in a similar energy range. From this we can conclude that the experience gained during the research can already be used to produce perovskites from the components of metal oxide. As a result, it can be a general mechanochemical perovskite synthesis model.

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ANALYSIS OF KINETICS OF POORLY WATER-SOLUBLE DRUG RELEASE FROM HYDROGELS BASED ON POLY(METHACRYLIC ACID) AND CASEIN WITH DIFFERENT CROSSLINKER AMOUNT

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Abstract

Nowadays, humanity are faced with many challenges which affect health of people all around the globe (such as climate change, new diseases and/or already present ones for which cure has not been found yet – cancer). The efforts of researchers on the field of drug delivery systems bring everyday novel tools for safer and more effective therapy. pH sensitive hydrogels based on poly(methacrylic acid) are recognized as materials with huge potential for controlled release of drugs. The encapsulation and controlled release of many chemotherapeutics is quite challenge due to their poorly water-solubility. In our previous research we overcome this problem by modifying hydrophilic pol(methacrylic acid) with amphiphilic casein and showed that prepared material have potential for encapsulation and controlled release of poorly watersoluble model drug - caffeine (PMAC carriers). In present study we deepened further our research and employed various models: Ritger-Peppas, Higuchi and Kopcha model to analyze how the change of crosslinker amount affect the mechanism of release kinetics of caffeine in medium with pH of 6.8 (which simulated the environment in human intestines). Obtained results showed that only by changing one parameter such as crosslinker amount it is possible to fine tune the type of drug release mechanism, due to which the PMAC carriers would be able to respond to the specific demands of therapy.

Introduction

Climate change and serious diseases are grate challenges of modern world. Researchers efforts in the field of medicine resulted in promising tools for safer and more effective therapy of serious diseases such as various types of cancer. One of these tools are certainly drug delivery systems which enable targeted delivery of a drug at specific place in human body and its controlled release due to which drug concentration remains constant. In that way bioavailability of drug is improved whereas side effects are reduced which further leads to the better therapy [1]. pH sensitive hydrogels based on poly(methacrylic acid) (PMAA) are materials with huge potential for controlled release of drugs. These hydrogels are non-toxic, biocompatible and are able to swell in environment with pH higher than pKa of PMAA and therefore release encapsulated drug in the process [2]. Due to the hydrophilicity of PMAA it is quate challenging to encapsulate poorly water-soluble drugs (such as many chemotherapeutics). We overcome this limitation by modifying PMAA with amphiphilic casein and demonstrated effectiveness of this carrier for encapsulation and controlled release of poorly water-soluble model drug caffeine (PMAC carriers) [2-5]. In our previous research we also showed that only by changing one synthesis parameter such as crosslinker amount it was possible to tune caffeine release profiles [5].

In present study we further deepened our research and employed several models (Ritger-Peppas [6], Higuchi [7] and Kopcha model [8]) to analyze how the change of crosslinker amount affect the mechanism of caffeine release from the PMAC carriers in phosphate buffer with pH of 6.8 at 37°C (PB 6.8).

Experimental

The PAMC carriers were synthetized by free-radical polymerization and all carriers had 4 ml of PMAA, 4 g of casein, 0.2 g caffeine and 0.9 ml of 1wt% aqueous solution of initatior (2,2)-azobis-[2-(2-imidazolin-2-yl)propane] dihydrochloride), whereas the amount of crosslinker - methylenebisacrylamide (MBA) was varried: 0.8mol%, 1.6mol% and 3.2mol% (with respect to the amount of methacrylic acid). The synthesis, the list of used chemicals and the feed composition are presented in our previous research [5]. The synthetized carriers were denoted as PMAC-xM, where xM represented the amount of crosslinker (M, 2M, 4M and 8M = 0.4 mol%, 0.8mol%, 1.6mol% and 3.2mol% of crosslinker, respectively).

In order to better understand the mechanism of caffeine release from the PMAC carriers and how the increase in the crosslinker amount affects it, caffeine release data were analyzed with the most commonly used models Ritger-Peppas (Eq. (1)), Higuchi (Eq. (2)) and Kopcha model (Eq. (3)):

$$\frac{M_t}{M_m} = kt^n \tag{1}$$

$$\frac{M_t}{M_{\infty}} = k_H \sqrt{t} \tag{2}$$

$$\frac{M_t}{M_{\infty}} = k_1 t^{0.5} + k_2 t^1 \tag{3}.$$

In all equations the $\frac{M_t}{M_{\infty}}$ represents the fractional drug release and t is the time of the drug release process (min). In Eq. (1) the coefficient k is the constant of the speed of drug release (min⁻¹) and exponent n shows the type of the mechanism of drug release (diffusion and/or relaxation of polymer's chains). In Eq. (2) the coefficient k_H represents the constant of the speed of drug diffusion from the carrier (min⁻¹). In Eq. (3) the coefficient k₁ is the constant of the speed of drug release governed by diffusion (min⁻¹), whereas the coefficient k₂ is the constant of the speed of the speed of drug release governed by relaxation of polymer's chains (min⁻¹).

The equations Eq. (1) and Eq. (2) were used in following forms: $\ln(\frac{M_t}{M_{\infty}}) = \ln(kt^n)$ and $\frac{M_t}{M_{\infty}} = k_H t^{0.5}$, respectively. The form of Eq. (3) was added into the OriginPro 8.5 program and then it was applayed on the caffeine release profiles $(\frac{M_t}{M_{\infty}} - t)$. The "fields of applicability" of applied models were denoted as $\Delta \alpha$ (%).

Results and discussion

The profiles of caffeine release in PB 6.8 were fitted with Ritger-Peppas (R-P), Higuchi and Kopcha model and are presented in Fig. 1., Fig. 2. and Fig. 3., respectively. Calculated kinetics parameters of applied models and corresponding fields of applicability are presented in Table 1. The analysis of the kinetics of caffeine release from PMAC-M were presented in our previous research [2], and only obtained values of kinetics parameter are presented in Table 1. in order to compare with the results obtained in present study.

R-P model showed that caffeine release from PMAC-M and PMAC-2M was governed only by relaxation of polymer's chains of the carrier's network, whereas caffeine was released from PMAC-4M and PMAC-8M carriers by both mechanism – diffusion and relaxation of polymer's chains. The obtained values of Higuchi coefficient k_H for all the samples were higher than the values of corresponding Kopcha coefficient k_1 , and the fields of applicability of these two

models were similar. However, Higuchi model does not consider the impact of the polymer's chains relaxation on the process of drug release. The increase in the crosslinker amount led to the decrease of the speed of caffeine release (decrease of the Kopcha coefficient k_2).



Figure 1. Fitting of the profiles of caffeine release from the PMAC carriers in PB 6.8 with R-P model









Figure 3. Fitting of the profiles of caffeine release from the PMAC carriers in PB 6.8 with Kopcha model

Table 1. Obtained kinetics	parameters of chosen	models for each PMAC carrier
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Model	Sample	PMAC-M	PMAC-2M	PMAC-4M	PMAC-8M
R-P	n	1.0	1.0	0.65	0.64
	$k*10^2$ (min ⁻¹)	0.930	0.648	2.97	3.14
	Δα(%)	71.5	59.1	69.8	77.1
	\mathbb{R}^2	0.911	0.965	0.988	0.984
Higuchi	$k_{\rm H}*10^2$	5.13	6.48	6.42	5.89
	Δα(%)	90.5	55.3	86.2	77.1
	\mathbb{R}^2	0.985	0.965	0.989	0.996
Kopcha	$k_1 * 10^2$	2.40	3.35	4.30	4.21
	$k_2 * 10^3$	4.52	3.55	2.07	1.81
	Δα	71.5	83.6	81.9	69.8
	\mathbb{R}^2	0.989	0.969	0.977	0.990

The fitting of the caffeine release data was the best with Kopcha model, therefore it can be concluded that the caffeine release from PMAC carriers was governed by both mechanism - diffusion and polymer's chains relaxation.

Conclusion

In our previous research we synthetized PMAC carriers and investigated how the increase in the crosslinker amount affect the swelling of the carriers and release of poorly water model drug - caffeine. In present study we did step forward and analyzed the mechanism of caffeine release from PMAC carriers in PB 6.8 and investigated how the change in crosslinker amount affect the type of the drug release mechanism. Several models Ritger-Peppas, Higuchi and Kopcha model were used for the analysis of kinetics of caffeine release from PMAC carriers. Obtained results showed that the best fitting of the caffeine release data was with Kopcha model and both mechanism (diffusion and relaxation of polymer's chains) governed the caffeine release from the PMAC carriers.

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NON-CHEMICAL CONTROL MEASURES OF Senecio vulgaris L.

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Abstract

Environmental constraints of crop production systems have stimulated interest in alternative weed management strategies, as the continued use of synthetic herbicides has resulted in serious ecological problems, such as weeds resistance to important herbicides and increased environmental pollution and health hazards. The aim of this study was to test the bioherbicidal activity of the essential oil of *Rosmarinus officinalis* L. on weed species *Senecio vulgaris* L., in 2019, at the Faculty of Agriculture, University of Novi Sad. In order to examine a phytotoxic effect, plants were treated with 1 and 5% (v/v) concentration of rosemary essential oil, kitchen salt NaCl (1:8), wine vinegar solution (1:10), and glyphosate, while the control variants remained untreated. The evaluation was carried out 1, 24, 48, 72, 96, 120 and 144 hours after application. Essential oils of rosemary in both concentrations and wine vinegar solution (1:10) had good efficacy compared to the control. High efficacy was in the treatment with NaCl solution and glyphosate, and complete decay of *S. vulgaris* plants occurred 144h after application. Phytotoxic changes in the form of turgor loss, chlorotic, then necrotic spots, were determined on the tested weed species 24h from the application of NaCl and glyphosate, while from essential oil first symptoms have appeared after 48h.

Introduction

Potential damage to human health and to the environment from herbicides is considered a real problem which has stimulated interest in using plant-derived compounds as a natural herbicide. The use of chemical herbicides besides their ecotoxicological effect could induce the emergence of resistant weed populations [1], such as continuous use of glyphosate has made certain populations become glyphosate-resistant (Lolium rigidum L., Echinochloa crus-galli (L.) Beauv.) [2]. During the last 40 years, a number of researchers have highlighted the potential importance of natural plant products as herbicides [3]. Among natural plant products, volatile essential oil and their constituents have attracted much attention because their phytotoxicity has shown strong activity against weeds [4]. Essential oils are complex of volatile compounds, naturally synthesized in different plant parts during the process of secondary metabolism [1]. Terpenoids, especially monoterpenes and sesquiterpenes, are the main components of essential oil and are often responsible for their inhibitory activity in plants. Tworkoski (2002) [5] demonstrated that essential oils from red thyme, summer savory, cinnamon, and clove were the most phytotoxic to Chenopodium album, Sorghum halepense and Ambrosia artemisiifolia. Herbicides based on plant essential oil have been demonstrated to be effective against a wide range of weeds and are potentially a natural alternative to non-selective herbicides [4]. Essential oils from species such as oregano, thyme, rosemary, sage and mint are reported to be particularly strong bioherbicide candidates [6]. Many recent studies have investigated the phytotoxicity of Rosmarinus officinalis L. extracts to weeds, and in this respect, significant results were obtained on a number of important weed species, such as Amaranthus retroflexus L., Bromus tectorum L., Cynodon dactylon L., Digitaria sanguinalis L. and Lolium perenne [7]. The aim of this study was to determine the influence of chemical and alternatives measures in the control of common groundsel (Senecio vulgaris L.).

Experimental

The plants of *Senecio vulgaris* L. were collected from an old vineyard and transplanted into containers. The experiment was set in six repetitions, and plants were grown in controlled conditions at the Faculty of Agriculture, University of Novi Sad during 2019. Treatments were done in the phase of 4-6 leaves, with a solution of rosemary essential oil in a concentration of 1% (v/v) and 5% (v/v), solution of wine vinegar (1:10) and kitchen salt NaCl (1:8). Plants were also treated with the non-selective herbicide glyphosate (3 l/ha), with the addition of 0.1% surfactant Trend 90. Untreated plants were also included in the experiment. The solutions were applied with hand sprayer Einhell BG-PS 1.5/1, after which the plants were placed in an air chamber with an average daily temperature of 25 °C. Evaluation of the effects of the examined treatments on whole plants was done by visual assessment according to EWRC (European Weed Research Council) scale from 1 (0% damage) to 9 (100% plant decay) [8], while for leaf damage a visual assessment was used according to a scale from 1 (0% damage) to 5 (100% plant decay) [9].

Results and discussion

The effects of rosemary essential oil in the tested concentrations (1 and 5% v/v), kitchen salt NaCl (1:8), wine vinegar (1:10) and glyphosate on the weed species *S.vulgaris* L. are presented in Tables 1 and 2. The evaluation was carried out 1, 24, 48, 72, 96, 120 and 144 hours after application.

Time of	Concen	tration	NaCl	Wine	Glyphosate	Control
assessment	of essental oil		(1:8)	vinegar	• •	
	of rosemary			(1:10)		
	1%	5%				
1h	1	1	4	1	1	1
24h	2	4	7	4	4	1
48h	3	4	8	6	6	1
72h	5	6	8	6	6	1
96h	6	7	9	7	7	1
120h	6	7	9	7	8	1
144h	7	7	9	7	9	1

 Table 1. Assessment of plant injury, EWRC scale

Table 2. Assessment of leaf injury, Gar scale

Time of	Concent	ration	NaCl	Wine	Glyphosate	Control
assessment	of essental oil		(1:8)	vinegar		
	of rosemary			(1:10)		
	1%	5%				
1h	0	0	1	0	0	0
24h	2	2	3	2	3	0
48h	2	2	4	2	3	0
72h	2	3	4	3	4	0
96h	2	3	4	3	4	0
120h	3	4	5	3	4	0
144h	4	4	5	3	5	0

One hour after treatment there were no signs of damage on plants from the applied concentrations (1 and 5%) of rosemary essential oil. After 24h and 48h at a concentration of

1% the plants were partial lodgings down (categories 2 and 3 on EWRC scale), while at a concentration of 5% they lay down (category 4 on EWRC scale), and chlorotic and necrotic spots were observed (category 2 on Gar scale). In the treatment with both concentrations of essential oil, after 72 and 96 hours there was the lodging of plants and color loss (category 5-7 on EWRC scale, 2 and 3 on Gar scale), while after 144h a more pronounced lodging was observed (7 on the EWRC scale), plants reacted by loss of turgor and color, chlorosis and necrosis (4 on Gar scale). Atak et al. (2016) [10] showed that concentrations of rosemary essental oil from 2 to 16 µl/Petri dish caused a decrease in germination from 61 to 12% for Avena sterilis L. seeds, while at least 2 µl/Petri dish inhibited germination of Sinapis arvensis L. by 89%. High efficacy was observed in the treatment with NaCl after 48 and 72h (8 on EWRC scale), where seedlings lay down, and strong chlorosis and color loss occurred (4 on Gar scale). Later, treatment with NaCl caused complete decay of S. vulgaris (category 9 on EWRC and 5 on Gar scale), which was manifested by turgor loss and necrosis. In the treatment with wine vinegar (1:10) the damage was tolerable to strong (6 and 7 on EWRC scale), with the appearance of chlorosis (3 of Gar scale). After 24 and 48h from the glyphosate application, there was the lodging of plants (4 and 6 on EWRC scale) and color loss (3 on Gar scale). Glyphosate achieved high efficacy after 144h, where the plants were completely dead (9 on EWRC scale), the leaves were chlorotic, necrotic, or fallen off (5 on Gar scale).



Figure 1. Average height and weight of S.vulgaris plants (after treatment and in control)

Six days after treatments, the average height and weight of S. *vulgaris* L. were measured. All treatments had an effect on the height and weight of the plants. The lowest average plant height was after the application of NaCl and glyphosate (fig. 1). According to the data obtained from Figure 1, it can be seen that rosemary essential oil of 1 and 5% (v/v), NaCl, wine vinegar and glyphosate had an effect on plant weight compared to control. The major effect on plant weight was observed with NaCl solution and glyphosate.

Benvenuti et al. (2017) [4] showed that the essential oil of *Xanthium strumarium* has an effect on reducing plant fresh weight of *Amaranthus retroflexus* and *Setaria viridis*, as well as on chlorophyll content, which confirms the total and rapid effectiveness of essential oil.

Conclusion

Essential oil of *Rosmarinus officinalis* L. at concentrations of 1 and 5% (v/v) showed efficacy in controlling Senecio vulgaris L. Good efficacy of essential oil was achieved 144 hours after

treatment, and it was manifested by turgor loss, color loss, chlorosis and plant necrosis. High efficiency of NaCl (1:8) was achieved after 48 hours, while complete decay of plants occurred 96 hours after application. Slightly lower efficacy was observed with wine vinegar (1:10), where the damage was tolerable to strong. Glyphosate showed high efficacy after 72h, and complete decay of *S. vulgaris* plants occurred 144h after application. Of all applied treatments, the highest efficacy was observed with NaCl and glyphosate, but the applied essential oil also had good efficacy in controlling *S. vulgaris*. After application, all treatments affected the height and weight of the plants. Due to the risk of possible resistance and questionable authorization of glyphosate after 2022, some possible alternatives should be considered for weed control.

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THE SOLUTION CHEMICAL BEHAVIOUR OF OXORHENIUM(V) AND HALF-SANDWICH RUTHENIUM(II) COMPLEXES CONTAINING A TRIPHENYLPHOSPHINE LIGAND

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Abstract

In the chemotherapeutic therapy of cancer there is an urgent need for the novel compounds, which have higher selectivity toward cancer cells and their use is accompanied with milder side-effects. In the field of inorganic medicinal chemistry a plethora of metal complexes with promising *in vitro* cytotoxic behavior was developed containing various metal ions (*e.g.* Cu(II), Au(III), Ru(II/III)) [1]. Complexes of Re(I) and Re(V) showed anticancer properties, and the latter group is able to cleave DNA [2]. Moreover, oxorhenium(V) complexes are catalyzing oxygen atom transfer reactions [3], which may occur intracellularly as well. The organometallic half-sandwich Ru(II) complexes containing cyclopentadiene (Cp) and triphenylphosphine (PPh₃) ligands were reported to exhibit anticancer activity *in vitro* and *in vivo* [4].

In this work I will introduce the different behavior of the PPh₃ containing Re(V) and Ru(II) complexes in solution (structures are shown in Figure 1.a,b). Synthesis and characterization of the complexes were followed and checked by ³¹P and ¹H NMR spectroscopy. For a RuCp complex, a single-crystal was also obtained which was suitable for X-ray diffraction analysis (Figure 1.c).



Figure 1. General structure of the studied a) RuCp and b) Re(V) complexes. c) ORTEP view of [RuCp(4,4'-dimethoxy-2,2'-bipyridine)(PPh₃)](CF₃SO₃)] complex.

Since phosphines are generally oxygen-sensitive, oxidation of the coordinated PPh₃ ligand was followed by ³¹P NMR spectroscopy. The compounds were dissolved in different organic solvent-water mixtures and their stability in solution was monitored by UV-visible spectrophotometry and ¹H and ³¹P NMR spectroscopy as well. The two complex families displayed remarkable differences in their solution chemical properties.

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ENVIRONMENTALLY FRIENDLY METHOD FOR S, N FUNCTIONALIZATION OF GRAPHENE QUANTUM DOTS

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Abstract

Graphene quantum dots (GQDs) show exceptional optical and physical properties. To enable their wider application and improve their optical properties, it is usually necessary to modify them with different functional groups and heteroatoms. Toxic reagents that are potentially harmful to human health and the environment are often used for these procedures. One of the methods that have been successfully used is gamma radiation. In this study, gamma irradiation was employed to achieve structural modification of GQDs without the usage of reactive, toxic chemicals. In this way, an eco-friendly and simple procedure for the incorporation of S and N atoms into the GQDs structure is developed.

Introduction

Graphene quantum dots (GQDs), a novel kind of nanomaterials that combines the characteristics of both graphene and quantum dots, have great potential as a luminescent material. GQDs consist of one or a few graphene layers with a lateral size of less than 100 nm [1, 2]. They are highly luminescent, biocompatible and dispersible in a variety of solvents which indicates that they have a great potential for use in bioimaging [3, 4], photovoltaic [5], sensing and light-emitting applications [6]. The excellent solubility of GODs in water and polar organic solvents is the result of hydrophilic functional groups (such as carboxyl, carbonyl, hydroxyl, and epoxy) bonded to the edges and basal plane of GQDs [7]. It has been shown that heteroatom doping can impact the intrinsic electronic and optical properties as well as surface reactivity of carbon nanomaterials [8]. This is an efficient technique to adjust the bandgap, modify the chemical activity and electronic density of GQDs, resulting in improved optical properties and wider application [9]. To achieve the structural modification of GQDs, chemically hazardous chemicals are usually used. As a result of that, waste and environmentally toxic chemicals are produced. One of the chemically clean and simple methods for changing the characteristics of graphene nanomaterials is gamma irradiation. It can be performed under different conditions and using different irradiation media. Besides irradiation conditions and medium, the effect of gamma radiation also depends on the type of irradiated material [10, 11].

Here, we proposed a new and simple method for the modification of GQDs without the usage of any hazardous chemicals. The functionalization was achieved by using gamma irradiation in the presence of L-cysteine as a donor of S and N heteroatoms and IPA alcohol as a radical scavenger. The experiment was performed in an oxygen-free atmosphere and the irradiation dose was 25 kGy.

Experimental

The method used for the synthesis of GQDs is the electrochemical oxidation of graphite electrodes. Water dispersion (1 mg/ml) of GQDs with 2wt% L-cysteine then was irradiated to incorporate S and N heteroatoms in the structure. The reduction atmosphere was achieved using water and isopropyl alcohol as a medium. Before irradiation, the mixture was purged with Ar gas to remove the oxygen molecules. The applied dose of gamma irradiation was 25 kGy. After

irradiation, the sample was dialyzed and evaporated to dryness. The collected powder was used for further characterization, labeled in this paper GQD-cys-25.

Absorption measurements were performed using GBC Cintra 6 spectrophotometer (GBC Dandenong, Australia) and the obtained UV-Vis spectra were recorded in a range from 200 to 800 nm. The concentration of the sample was 0.25 mg/ml and the measurements were carried out in the air environment and room temperature.

Atomic Force Microscopy (AFM) was performed using a Quesant microscope (Agoura Hills, CA, United States), operating in a tapping mode. Water dispersions of the samples in a concentration of 0.25 mg/ml were sonicated for 30 minutes and then spin-coated at 3500 rpm for 60 seconds on mica substrate. For image processing, Gwyddion 3.5 software was used.

Scanning electron microscopy (SEM) with energy-dispersive X-ray (EDS) spectroscopy was conducted on an FEI ESEM Quanta 200 microscope (FEI Company, Hills-boro, OR, Unites States). The samples were dispersed in ethanol, then drop-casting onto clean Si-substrates (10x10 mm) and dried overnight under the vacuum. EDS measurements were made by focusing the analysis across a 230x200 micron region and the obtained data was conducted with EDAX Genesis EDS microanalysis software (AMETEK, Inc.).

Thermo Scientific Nicolet 6700 FTIR instrument (Thermo Fischer Scientific, Waltham, MA, United States) was used to obtain FTIR spectra in attenuated total reflection (ATR) mode. The wavelength range was from 900 to 4000 cm⁻¹ and all samples were in powder form.

Results and discussion

UV-Vis spectroscopy was used to determine the optical properties of the samples and the obtained results are presented in Figure 1a.



Figure 1. a) UV-Vis spectra and b) FTIR spectra of p-GQDs (black line) and GQD-cys-25 (red line)

In UV-Vis spectra (Figure 1a), the absorption band at around 200 nm was detected and assigned to π - π * transitions due to the presence of C-C bonds. For the GQD-cys-25 sample, this band has been shifted to the lower wavelength which can be attributed to the modification in the structure of GQDs.

FTIR spectra (Figure 1b) show the successful binding of S and N atoms in the structure of gamma-irradiated GQDs. Both p-GQDs and GQDs-cys-25 show the peak at around 3400 cm⁻¹ which stems from the vibrations of OH groups while only the irradiated sample showed the peak at 3250 cm⁻¹ which originates from N-H stretching vibrations [12]. This peak as well as the peak at around 1700 cm⁻¹ proves the successful incorporation of N heteroatoms in the GQDs structure [13]. The band at 1600 cm⁻¹ proved that the aromatic structure in the irradiated sample was preserved after functionalization. The successful binding of S heteroatom was proved with

a peak at 1250 cm⁻¹ which appeared only in the gamma-irradiated sample and it is assigned to the C=S stretching vibrations from the thiocarbonyl groups [14].

The chemical composition and morphology of p-GQDs and GQD-cys-25 were investigated with SEM-EDS analysis (Figure 2).



Figure 2. SEM images and EDS spectra of p-GQDs (a, b) and GQD-cys-25 (c, d).

The grainy surface was present in both samples (Figure 2a and c). The elemental content investigated with EDS showed the presence of sulfur only in the irradiated sample. The atomic percentage of sulfur was 1.91 at%. The successful incorporation of S into the structure of GQDs using gamma irradiation was once again confirmed.

Morphology of GQDs before and after irradiation was investigated using an AFM microscope. The obtained results are presented in Figure 3.



Figure 3. AFM images for p-GQDs (a) and GQD-cys-25 (b).

Both images showed uniform particle-like morphology of GQDs. The average diameter for p-GQDs was 20.4 nm and for irradiated dots was higher, 25.5 nm. The average height was 2.6 nm and 1.6 nm for p-GQDs and GQD-cys-25, respectively. It was concluded that after gamma irradiation the particle diameter increases while at the same time their height decreases.
Conclusion

The proposed method for structural modification of GQDs using gamma irradiation allows avoiding the use of harmful and toxic chemicals, which contributes to the overall reduction of environmental pollution. This research aimed to successfully incorporate sulfur and nitrogen heteroatoms into the structure of GQDs. For this purpose, gamma irradiation was used in the presence of the amino acid L-cysteine and isopropyl alcohol as the reaction medium. The characterization of obtained material confirmed the presence of both heteroatoms, S and N. By employing the suggested method, the functionalization of GQDs was successfully achieved while avoiding the use of toxic and harmful chemicals.

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THERAPEUTIC PROPERTIES AND PHENANTRENS COMPOUNDS OF TAMUS COMMUNIS

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Abstract

From ancient in folk medicine the plants were used with lots of trust and efficiency. In time, people learn how to cultivate, to sample, and preserve different anatomical parts of the plant for medical purposes. Thus, also *Tamus communis* became an interesting plant, being used in different pharmaceutical formulations to treat different medical conditions. The extracts of root of *T. communis* is just one phyto-pharmaceutical form that demonstrated to have anti-inflammatory and antioxidant effect. Different anatomical parts of *T. communis*, various extraction methods using various solvents, were used in experimental researches to test the efficiency as cytotoxic and antiviral activities, antioxidant activity, anti-inflammatory and analgesic activity. Extracts of *T. communis* are used for preparation of poultice, ointments, and also for infusions, tincture preparation with very good pharmacologic activity. This paper only made a very succinct presentation of the possibilities to used *Tamus communis* as a main ingredient of different natural pharmacological preparation with applicability in human and veterinary medicine.

Introduction

Since ancient many researchers and medical doctors encourage the use of medicinal herbs to prevent, treat and preserve health conditions. Plants – through the active principles contained – can be used as ingredients of pharmaceutical preparations with beneficial effects on human health and not only, but also can be used as ingredients of natural nutritional supplements. People are always looking for organic, natural alternatives that bring health-promoting properties and look back in time to find out more details about specific plants that were used as different products for various medical purposes.

One of the flowering plant is *Tamus communis*, known also as *Dioscorea communis* or black bryony, whuch is used for its anti-inflammatory and antioxidant properties. The plant contains also saponins – and because of this fact – its use in different pharmaceutical forms

has to be very well documented, and also theoretical compositional and experimental evaluated [1].

Experimental studies showed that it is very important the time of the sampling, and more than this is the anatomical part that it is therapeutically used.



Regarding the anatomical part of the *Tamus communis* we have to underline the rhizomes which contain calcium oxalate and histamines which – in high concentrations – may cause skin irritation and dermatitis.

In Romania, black bryony is known in the folk area as "earth butter" and is described as an herbaceous plant, which has a stem of 2-4 meters long clinging on other plants or on stones. Sometimes it is also named as "black vine", because it develops a thick root up to half a meter long. In Romania *Tamus communis* it grows spontaneously in the sub-Carpathian area in deciduous forests, in shady and stony places, where its root hides being difficult to remove. This plant is used in traditional medicine, being an old remedy for locals. Its root (rhizome) is fleshy, presenting a white core, hence its name as "butter", and the popular name is more than suggestive in terms of the therapeutic role of the plant "grass of beaten women", because it was used to treat trauma and rheumatic pain, but also to strengthen weakened bodies [2].

Chemical composition

The chemical composition of *Tamus communis* varies by the anatomical part, but the plant contains alkaloids, saponins histamine, flavonoids, allantoin and tannins; vitamins A, vitamin C, vitamins from B complex; mineral salts of calcium, magnesium, sulfur, manganese, zinc (represented very well by calcium oxalate); mono- and polyunsaturated fatty acids.

Experimental studies demonstrated that *T. communis* contain different flavonoids, glucans, sterols, phenanthrene derivatives, spirostane and fructosane derivatives.

Chloroform (CHCl₃) extracts of the rhizomes of black bryony identified several phenanthrene (figure 1) such as: 2,7,8,-trimethoxy-3,4-methylen-dioxy-phenanthrene (compound 1 - c1), 7-hydroxy-2,8-dimethoxy-3,4-methylen-dioxy-phenanthrene (compound 2 - c2), 8-hydroxy-2,3,4,7-tetra-methoxy-phenanthrene (compound 3 - c3), 7-hydroxy-2,4,6-tri-methoxy-phenanthrene (compound 4 - c4), 4,7-dihydroxy-2,3-di-methoxy-phenanthrene (compound 5 - c5), and 4,8-di-hydroxy-2,3,7-tri-methoxy-phenanthrene (compound 6 - c6), respectively [3,4,5].

R ³ R ² R ¹	Specifi- cation	R	R1	R2	R3	R4	R5
	c1	-OCH ₃	-O-CH ₂ -O	-H	-H	-OCH ₃	-OCH ₃
4	c2	-OCH ₃	-O-CH ₂ -O	-H	-H	-OH	-OCH ₃
$n \overline{\gamma}$	c3	-OCH ₃	-OCH ₃	-OCH ₃	-H	-OCH ₃	-OH
8 14 11	c4	-OCH ₃	-H	-OCH ₃	-OCH ₃	-OH	-H
	c5	-OCH ₃	-OCH ₃	-OH	-H	-OH	-H
R ² 9 10	c6	-OCH ₃	-OCH ₃	-OH	-H	-OCH ₃	-OH

Figure 1. Phenanthrene compounds determined from Tamus communis solvents extracts

Black bryony contain phenolic compounds such as: quercetin-O-rhamnosyl-O-rhamnoside and kaempferol-3,4'-di-O-rhamnoside in significant quantity compared to *Lonicera periclymenum* and *Bryonia dioica*, but low quantity of cis-5-O-caffeoylquinic acid and trans-3,5-O-dicaffeolyquinic acids; and flavones represented by apigenin-6-C-g|ucoside-8-C-g|ucoside [6].

From literature data we can observe that the extracts rich in phenolic compounds of *Tamus communis* are often related with good bioactivity, which is associated with antioxidant activity, mainly of hydro-soluble gels formulations.

Phytotherapy based on Tamus communis

The active principles of *Tamus communis*, represented by phenolic acids, flavonoids, anthocyanins, and phenanthrene compounds which are mostly used as hydro-soluble extracts and gels for topical applications [6,7].

Anatomical parts of *T. communis* contain also steroidal saponins – which can be used as precursors or raw material for steroid hormones synthesis – compounds used in contraceptive preparations [8]. Also, it is not recommended the consumption of raw *T. communis* due to the content of different saponines and other compounds which are toxic, especially in significant quantities, so it is not recommended to be used as food or to be ingested at all!

Fresh root can be used in preparation of poultice which is recommended for inflamed joints and bruises.

The root of *T. communis* is used for preparation of infusion or cold maceration with benefic effect when used as local compresses.

The tincture is used in the form of compresses or local rubs.

The ointment from the earth butter is used in the form of massage or external applications in case of rheumatic pains, bruises, bruises, contusions.

Natural therapy with black bryony showed good effects in cases of rheumatism, gout, osteoporosis, lumbosciatica, cervical spondylosis, acute or chronic meniscus pain, shingles, frontal or maxillary sinusitis, arthritis, trauma, hematomas, frostbite, bone deformities, osteoarthritis (hip osteoarthritis, gonarthrosis), purple dermatoses, and acne [9, 10, 11].

Phytotherapy with black bryony products has allergenic, analgesic, anti-inflammatory, antirheumatic action, decongestant, diuretic, histaminic, emetic, laxative, rubefacient toxic, propecic, vulnerary, similar to chemically synthesized anti-inflammatory drugs (but some of them has side-effects), venous tonic action, stimulates local circulation, easily revulsive action. Phenanthrene derivatives presented cytotoxic and antiviral activities, while flavonoids from black bryony presented significant antioxidant activity [12, 13, 14, 15; roots aqueous and ethanol extracts showed anti-inflammatory and analgesic activity [16, 17].

Team work of Kucukboyaci evaluated the antioxidant and antimicrobial activity of aqueous extracts of *T. Communis* [18, 19]. Thus, they made two aqueous extracts of aerial parts with distillated water, and then lyophilized the extracts. Further, they made ethanol, *n*-hexane, ethyl acetate and chloroform separated extracts with the lyophilized plant material, and after they used those extract to evaluate the antioxidant activity by flow injection analysis and thiobarbituric acid assay. The aqueous plant extract was able to concentration-dependent scavenge DPPH radicals, where the quercetin was the most effective, followed by ascorbic acid, and then butylated hydroxytoluene (BHT). Also, phenolic and flavonoids compounds were reported having responsible for antioxidant potency of the *T. communis*. The experimental tests for antimicrobial activity demonstrated that the extracts did not present significant antimicrobial activity. Also, the concentration of minerals was low for potential and toxic elements such as: lead, cadmium, aluminum, but presented moderate concentrations of calcium magnesium, copper, iron, manganese and zinc [20].

Due to the oxalates crystals in needle shapes, some patients can also present histaminic reactions, and also by ingestion it could be possible to irritate the oral cavity, esophagus, stomach, and intestines.

The ethanol extracts of *T. communis* (80% ethanol, Soxhlet extraction) was used for impregnation of sterile cotton pellets, and after impregnation the ethanol was extracted by evaporation under vacuum using a rotavapor. The cotton pellets with plant bioactive compounds were used for determination of anti-inflammatory activity. The results proved that granuloma formation was decreased by 12, 27 and 49% for the dose levels of 5, 10 and 20 mg/pellet [17].

Conclusions

Tamus communis is a plant that we can found in different geographical areas. It is used mostly for preparation of extracts (aqueous, chloroform, ethanoic or other solvent extraction), poultice, infusions, tincture or ointments.

The anatomical parts of the black bryony present different composition in phenanthrens, phenols, flavones, anthocyanins, and minerals.

Pharmaceutic forms containing active principles of *T. communis* are used for as natural therapeutic approach which showed good effects in cases of gout, osteoporosis, cervical spondylosis, lumbosciatica, acute or chronic meniscus pain, rheumatism, shingles, frontal or maxillary sinusitis, trauma, hematomas, frostbite, arthritis, bone deformities, osteoarthritis, purple dermatoses, and acne.

Because of this benefic effects the plant is valuable as phyto-therapeutic natural product and deserve more experiments using different anatomical parts, various extraction solvents and methods, and more experimental tests for many disorders and medical problems.

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GAMMA IRRADIATION AS A TOOL FOR MODIFICATION OF GRAPHENE OXIDE-SILVER NANOWIRES COMPOSITES

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Abstract

Graphene oxide (GO) was produced using the Hummers' method while silver nanowires (AgNWs) were obtained by polyol synthesis. Composite was produced by simple mixing of GO and AgNWs dispersions. The composite was produced in a form of free/standing films by vacuum filtration and exposed to gamma irradiation in an oxygen-free atmosphere. After irradiation, without any additional cleaning, the structure, morphology and electrical properties were investigated. Gamma irradiation was shown to be an efficient tool to induce a chemical reduction of GO, and it was able to improve the electrical conductivity of produced composites. Due to avoiding the usage of reagents and solvents, this method belongs to green chemical approaches.

Introduction

Graphene oxide (GO) is a water-dispersible derivate of graphene [1]. While graphene is built from only benzene aromatic rings, it is highly hydrophobic and hard to disperse in polar organic solvents and water, GO has a large amount of polar, O-containing functional groups in the amount of 33.6 at% [2], made GO dispersible in water and polar solvents. On the other side, when O-functional groups are incorporated into graphene sheets, they disrupt the delocalized π -electronic cloud, responsible for extraordinary electrical properties of graphene such as electrical conductivity, and increased the resistance of 14.8 M Ω [2]. Thus, GO is more processable than graphene, but it does not have graphene's electrical properties.

Due to this condition, GO was often reduced using different chemical reactions, such as reaction with hydrazine monohydrate, H_2 , sodium borohydride, or HI [2-5]. These reactions demand the use of dangerous, hazardous reagents as well as organic solvents to remove residual reagents and side products. Due to the price of chemicals and appropriate handling and storing of toxic waste, these procedures are both ecologically and economically unfavorable.

Herein, we investigated the possibility of using gamma irradiation in the reduction of GO in a composite based on GO and silver nanowires (AgNWs). These composites attract scientific attention due to their good electrical conductivity and electromagnetic shielding efficiency [6-8]. Studies have shown that the electrical properties of GO-AgNWs can be improved by chemical reduction. In this paper, we explored the effects of different doses of gamma irradiation onto GO-AgNWs under an oxygen-free atmosphere. Gamma irradiation of GO at a dose of 35.3 kGy was able to reduce the sheet resistance from $10^7 \Omega \Box^{-1}$ to $100 \Omega \Box^{-1}$ [9]. During the exposure, gamma rays induce radiolytic decomposition of the medium and, if oxygen is present, oxidative, oxygen-containing free radicals species were produced and caused the oxidation of the material [10], while in the case when the medium for irradiation is oxygen-free or contained quenchers of oxygen free-radicals [11, 12], the chemical reduction will occur.

Thus, in this study we selected doses of gamma irradiation up to 35 kGy and an oxygen-free atmosphere.

Experimental

GO was produced using a previously described modified Hummers' procedure [13]. AgNWs were obtained according to the polyol method [14]. Composite GO-AgNWs were prepared by simply mixing the same volumes of GO dispersion in water (1 mg mL⁻¹) and AgNWs (1 mg mL⁻¹) in ethanol. A magnetic stirrer was used for homogenization and after 1 h of mixing, the dispersion was deposited onto the membrane filter (*Isopore* membrane filter, polycarbonate, hydrophilic, pore size 0.22 μ m) using a vacuum filtration system. The composite was peeled off from the membrane after drying, and collected as a free-standing layer and named GO-AgNWs 1:1. The samples were then exposed to gamma irradiation. Argon atmosphere was selected as an irradiation medium. Samples were exposed to irradiation doses of 15, 25 and 35 kGy and named GO-AgNWs 1:1¹⁵, GO-AgNWs 1:1²⁵, and GO-AgNWs 1:1³⁵, respectively. Structure, morphology and electrical properties were investigated before and after gamma irradiation.

Quesant atomic force microscope (AFM, Agoura Hills, CA, USA) was used to investigate the morphology of films. It was operating in tapping mode, at room temperature. All measurements were done under the air atmosphere. Q-WM300, a monolithic silicon AFM probe was used. The non-contact mode was selected for obtaining the AFM images. We used standard silicon tips with a force constant of 40 N/m by NanoAndMore GmbH, Wetzlar, Germany. Gwyddion 2.53 software was used for image analysis [15].

Both GO and composite based on GO and AgNWs were investigated using scanning electron microscopy (SEM) with energy-dispersive X-ray (EDS) spectroscopy. The FEI ESEM Quanta 200 microscope (FEI Company, Hillsboro, OR, USA) was used. Free-standing films were scanned at the SEM microscope without any additional preparation of the surface of the sample (no metalization). A "low vacuum mode" was selected. EDS measurements were obtained focusing the analysis over an area of ca. 230x200 microns. Data were analyzed using the EDAX Genesis EDS microanalysis software (AMETEK, Inc., Berwyn, PA, USA).

Electrical properties of the material were investigated using a 4-point probe Jandel RM3000+ (Leighton Buzzard, UK) test unit. The probe spacing was 1 mm. Measurements were conducted in room conditions, each measurement was repeated at 3 different locations and the average values of sheet resistances and conductivities were calculated.

Results and discussion

Morphology of the composite before and after gamma irradiation was investigated using an AFM microscope and these results are presented in figure 1. It was calculated that the mean surface roughness was 376 and 210 nm for GO and GO-AgNWs 1:1, respectively, while for composites irradiated with a dose of 15, 25, 35 kGy, it was measured 237, 265, 219 nm, respectively.



Figure 1. AFM images of GO-AgNWs 1:1 (a), after irradiation at a dose of 15 (b) and 35 kGy (c).

SEM-EDS was used to investigate both the morphology and chemical composition of GO and composite (figure 2.). It was detected GO free-standing film has a uniform, grainy surface (figure 2a and b), while the cross-section image showed that the thickness was around 12 μ m. In the case of the composite, cylindrical objects were noticed (figure 2e and e), which were particularly clear at the cross-section image (figure 2g). The thickness of GO-AgNWs 1:1 was 17 μ m. EDS spectra showed that C and O were dominant in GO film, while C, O and Ag were detected in composites (figure 2d and h).



Figure 2. Top view (a, b) and cross-section SEM images of GO (c), and EDS spectrum of GO (d); and the same for composite GO-AgNWs 1:1: top-view (e, f), cross-section (g) and EDS (h), respectively.

In figure 3, photos of GO and GO-AgNWs 1:1 are displayed. As it can be seen, GO film was easily breaking upon bending while composite GO-AgNWs 1:1 was more elastic and mechanically stable.



Figure 3. Photos of the free-standing film: GO and GO-AgNWs 1:1, as indicated.

When the composite was exposed to gamma irradiation, the changes in electrical properties were investigated using a 4-point probe. In figure 4a, conductivity vs. applied gamma irradiation dose is reported. At the lowest applied dose (15 kGy), conductivity was significantly increased, from 167 to 430 S cm⁻¹. At higher doses, 25 and 35 kGy, conductivity also increased, for 99.4 and 32.9%, respectively. These results can be explained by taking into account the chemical reduction of GO during gamma-irradiation, which leads to an increase of conductive, π -domains in graphene sheets [16, 17]. The conductive nature of the gamma-irradiated composite with the dose of 15 kGy was studied by analyzing the current (I)–voltage (V) curve (figure 4b). The current increased linearly with voltage (figure 4b).



Figure 4. The conductivity of GO-AgNWs 1:1 vs. applied gamma-irradiation doses (a) and I-V curve for GO-AgNWs 1:1¹⁵ sample (b).

Conclusion

Although chemical reduction of GO and GO-based composites leads to improvements in the electrical conductivity of these materials, these procedures demand the usage of toxic and hazardous chemicals. Gamma irradiation showed to be a good alternative due to avoiding of usage of chemical reagents as well as no need for the cleaning stage. By simple gamma irradiation of free-standing GO-AgNWs composites under argon atmosphere, electrical conductivity increased by almost three times.

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SUGAR SUBSTITUTE COMPOUNDS AS ENVIRONMENTAL EXPOSURES – ASPECTS FROM NEUROENDOCRINE FUNCTIONS

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Abstract

It is an extremely interesting question to what extent elements of psychic activity (e.g. cognition) in social existence can change by mediating neuroendocrine communication when it is necessarily altered by chemical environmental influences (e.g. nutritional biological agents) through real technosphere exposures. In the processes of learning and memory, biological mechanisms regulated by the neuro-endocrine system appear predominantly, showing a network relationship with essential local system properties. Exposures (dietary supplements) are tested on in vitro models by monitoring the events of neuro-endocrinological communication (hormone secretions, monoamine /adrenaline and serotonine/) and their changes in exposure.

Introduction

The quality of nutrients is of particular importance in the elements of the environmental system. In normal human homeostasis, the carbohydrate, lipid, protein and mineral as well as vitamin content of nutritional ingredients is determinant in maintaining health. When some systemic human disorder, e.g. sugar metabolism occurs, the quality and quantity of these otherwise simple intakes also change [1]. It is important to declare that sugar is an inseparable part of the human nutrition, but because of the health some people choose to limit their food intake by replacing sugar with e.g. saccharin and stevia. The neuroendocrine system is of key importance in the regulation of metabolism, the elements of which can consequently regulate higher-order behavioural processes [2]. Modification of regulatory processes, e.g. through sugar replacement agents, can modify established equilibrium regulatory processes. Due to its anti-diuretic role, arginine vasopressin (AVP) is essential in osmoregulation, volume regulation, and is a carrier of information in behaviour, learning and memory functions [3]. This endocrine hormone is involved in neuronal processes via the monoamine signalling, which is confirmed by the mechanisms of e.g. serotonine (5-HT), adrenaline (E) [4, 5]. It is a very interesting question to what extent the psychic activity elements of social life (e.g. learning) can change by mediating neuroendocrine communication when it is necessarily altered by energy recovery routes (nutritional biological agents such as sugar substitutes). Energy generation mechanisms provide chemical energy transfers from the breakdown of raw materials (carbohydrates, lipases, nucleic acids, proteins) that are necessary to maintain healthy life phenomena. Regulatory systems with neuro-endocrino-immune functions control this large metabolic cascade. If the wrong material enters the metabolic pathway in a healthy organism, the regulatory cycles try to compensate for its disturbance [6]. All this can lead to a decrease in the complex homeostatic capacity, which in turn is accompanied by a loss of necessary adaptation possibilities.



Figure 1 Neuroendocrine regulation and possible endocrine disturbance by sugar replacement

Aims

In our research, we wanted to investigate this issue in relation to monoamine activated (neural transmitter functions) hormone regulations (AVP). We wanted to study the effects of sugar replacement on monoamine-mediated hormone release functions in an *in vivo and in vitro* animal model using a cellular research design.

Methods

In the experiment Wistar \bigcirc rats were treated *in vivo* with, saccharin: 0.2 mg/ bw.kg/day, and stevia: 40 mg/ bw.kg/day for 16 weeks (n=6/groups). After treatment, from the Wistar rats neurohypophysis were prepared for *in vitro* primer, monolayer cell culture model (NH). The tissues were digested enzymatically (trypsin: 0.2 % /Sigma, Germany/ for 30 min; collagenase /Sigma, Germany/: 30 µg/cm³ for 40 min; dispase /Sigma, Germany/: 50 µg/cm³ for 40 min in phosphate-buffered saline; temperature: 37°C). Mechanical dissociation was achieved with nylon blutex sieves (\emptyset : 83 and 48 µm). Cultures were controlled for viability (>95%) by trypan blue tests, after than for function for AVP release (in aspecific and specific regulation). In the research protocol was investigation in NH models: untreated as control, treated with 10⁻⁶M of 5-HT, 10⁻⁶M of E and then exposed to saccharin and stevia, during 120 min. The AVP releases of NH model were measured by radioimmunoassay. The protein content was detected by modified Lowry method. The data were analysed by ANOVA (n=5).

Results and discussion

In our results showed mild modified AVP release activity by different treatments of sugar substitutes.







Figure 3. The effects of *in vivo* Stevia treatment on E activated AVP release in NH cultures from Wistar rats (n=5, means± S.E.M., a: p<0.05)



Figure 4. The effects of *in vivo* Stevia treatment on 5-HT activated AVP release in NH cultures from Wistar rats (n=5, means± S.E.M., a: p<0.05)



Figure 5. The effects of *in vivo* Saccharin treatment on 5-HT activated AVP release in NH cultures from Wistar rats (n=5, means± S.E.M. a: p<0.05)

Conclusion

In conclusion sugar substitutes modulated (not significantly) the E and 5-HT-activated hormone secretion, which is definitely attention-raising. Our results indicate that a strict association exists among certain biophysical properties, especially the sugar substitutes and cellular function, this observation is essential because AVP can affect the learning processes. The potential endocrine modulating effect of fashionable sugar replacement compounds can certainly contribute to the modelling of the endocrine disrupting factor of the model developed by our working group.

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NON-AMBIENT FTIR STUDY OF THERMALLY TREATED SEASHELLS

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Introduction

A large quantity of waste seashells (millions of tons) is discarded annually after mollusc consumption, which becomes a problem for the environment worldwide as these shells are a habitat for microbes which could turn into a public health issue [1]. On the other hand, waste shell biomaterials need a small amount of energy for recycling and processing into useful products for various applications.

Calcium carbonate is essential in biomineralization, where it is the primary constituent of mollusc shells, crustacean cuticles, corals etc. In its pure form, it occurs naturally, under normal temperature and pressure conditions, in three anhydrous crystalline forms, namely calcite, aragonite and vaterite [2]. At high pressures, two further forms, namely calcite II and calcite III, are formed. The most stable form of calcium carbonate at atmospheric pressure and room temperature is calcite. The presence of both calcite and aragonite is very frequent in biologically produced calcium carbonate minerals. Though aragonite is metastable in aqueous solution, it can nucleate [3]. Aragonite is found in the nacre of the shells of bivalve molluscs, which provide a protection for these animals [4]. From the study of the marine bivalve species *Mercenaria mercenaria* and *Crassostrea gigas*, Weiner et al. [5] have shown that amorphous calcium carbonate is a precursor phase of aragonite.

The present study, which is part of our efforts to convert seashells into useful products like hydroxyapatite, investigates the temperature influence on the calcite and aragonite in a processed shell sample by using Fourier-transform infrared spectroscopy (FTIR).

Experimental

Seashells were thermally treated by using a Hygiena dry block incubator and closed glass tubes. First, the shell fragments used in these tests were mechanically processed to give a diameter between 1.0 and 1.6 mm. The Na₂HPO₄·12 H₂O reagent was used, giving a pH of 9.1, and the tube samples were kept at a temperature of 90 °C for 16 h. The 0.468 mol L⁻¹ Na₂HPO₄ concentration, in relation to the amount of shells used, should have led to a degree of conversion for calcium carbonate of maximum 5%. However, the target compound hydroxyapatite was not confirmed by XRD or FTIR.

FTIR investigations of the obtained products were performed by using a Vertex 70 (Bruker) FTIR spectrometer. In order to characterize the pre-treated material, the temperature dependence of spectrum change in the middle infrared domain was studied in the 20–200 °C temperature range with 20 degrees increments. The triturated sample was incorporated in a 13 mm KBr pellet and subjected to a pressure of 9 kPa. For a better signal-to-noise ratio, 128 recordings were accumulated for each temperature.

Results and discussion

Although hydroxyapatite crystallization did not seem to occur, even at high temperatures, in our sample, and thus the compound was not confirmed in the tests we performed, some interesting observations were made regarding the FTIR spectra at different temperatures. In the 400–4000 cm⁻¹ spectral range, the characteristic bands of carbonate ion, mainly in calcite form, were identified (Fig. 1). The absorption band at 1466 cm⁻¹ corresponds to the antisymmetric stretching vibration of two C-O bonds whereas the band at 1080 cm⁻¹ can be attributed to symmetric stretching vibration of the same bonds. The absorption appearing at 861 cm⁻¹ is generated by the out-of-plane vibration of the whole carbonate anion and the band at 712 cm⁻¹ is due to the antisymmetric deformation of the angle between two C-O bonds.



Figure 1. Middle infrared spectrum of shell sample at 20 °C

The intensity variations of some selected absorption bands exhibit particular regularities vs. temperature in selected wavenumber ranges. The absorption in the $3200 - 3600 \text{ cm}^{-1}$ domain is characteristic to the hydroxyl group (Fig. 2). Regarding the dependence of the absorption intensity on the temperature, two temperature zones can be distinguished. Below 40 °C the intensity decreases drastically, but above this temperature there is a significantly moderate decrease, indicating a distinct event. Up to 40 °C, the residual moisture is removed from the sample, but above this value there is a process of elimination of the more strongly bounded crystallization water (Fig. 3). The heating effect upon the absorbance at 712 cm⁻¹, associated with in-plane asymmetric vibration (Fig, 4), exhibits a change of slope at 40 °C as well (Fig. 5).



Figure 2. Absorbance of the OH stretching vibration range vs. temperature



Figure 3. Absorption intensity vs. temperature at 3446.59 cm⁻¹



Figure 4. Absorbance in the range 680 - 740 cm⁻¹ vs. temperature



Figure 5. Absorption intensity vs. temperature at 712.65 cm⁻¹

Further remarkable slope breakings, regarding the absorbance vs. temperature, are found for the absorption bands placed at 1458.10 cm⁻¹ (Fig. 6) and 1506.32 cm⁻¹ (Fig. 7), but in these cases the slope break appears at 100 °C.



Figure 6. Absorption intensity vs. temperature at 1458.10 cm⁻¹



Figure 7. Absorption intensity vs. temperature at 1506.32 cm-1

Outlining an isosbestic point around wavenumber 1073 cm^{-1} seems to support this interpretation. Figs. 8 and 9 illustrate the bathochromic shift of the position of maximum absorption vs. temperature.



Figure 8. Bathochromic displacement of the absorption band in the range 1070 - 1090 cm⁻¹ as the temperature rises



Figure 9. Position of the maximum absorption in the spectral range 1080 - 1082 cm⁻¹

Conclusion

The behavior of the bands between $1070 - 1090 \text{ cm}^{-1}$ (Fig. 8) is more special in the sense that, as the temperature rises, their location changes progressively (Fig. 9), indicating the presence of at least two distinct phases in the sample that gradually transform into each other.

It is important to notify the two values of temperature (slightly over 50 °C and over 100 °C) at which the sudden variations of the wavenumber values occur, somewhat consistent with the results in the Figs. 3, 5, 6 and 7.

Although hydroxyapatite was not confirmed in these experiments, it can be stated that the studied material presents at least two distinct phases in the considered temperature domain.

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SELENIUM IN FOOD AND FOOD SUPPLEMENTS

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Abstract

The bioelements detected in the lithosphere and biosphere have aroused major interest in geology and biology. It was detected in minerals but also in tissues taken from plants, animals and humans. Among the bioelements, the natural distribution of selenium (Se) was studied, following the quantity in food, the structural specificity of the components. Along with these, the biological activity was studied, following its integration in food supplements.

Key words: selenium in environment and foodstuffs; structure-activity relationship

Introduction

Selenium is a trace element occuring in all cells of the organism. Its quantity varies from tissue to tissue being dependent on the nutritional intake conditioned by the geographical environment (soil, water). Selenium can be found in higher concentrations in thyroid, kidneys, testicles, liver, etc.

It was identified in 1817 by Berzelius and considered long time as toxic. Only in 1957 was it recognized as an important micronutrient for the organisms.

1. Structural forms of selenium in the environment

In the environment, selenium is found in soils both in inorganic and organic forms. The oxidation states of selenium compounds range from -2 to +6. Its geographical distribution varies greatly, the amount of selenium in soil being between 0.01 - 2 mg/kg.

The inorganic form of Se in soil are: selenate (SeO_4^{2-}) - a compound with good water solubility and bioavailability; selenite (SeO_3^{2-}) – having a water solubility and bioavailability below that of selenate. Also, elemental selenium (Se^0) or selenides (Se^{2-}) can also be found in soils - these being insoluble forms of selenium. The organic forms of selenium found in soil include methylated or unmethylated selenium amino acids, dimethyl selenide, dimethyl diselenide, selenomethionine; selenocysteine [1, 2, 3]. In drinking water selenium can come from discharges from mines, natural deposits, dischage from refineries, or from agricultural activities. Also, selenium is found in foods of plant and animal origin [4, 5].

The selenium compounds found in foods are the amino acid derivatives selenomethionine, Semethylselenocysteine, selenocysteine. The inorganic forms selenite and selenate are found in smaller amounts (fig.1).



There are 25 selenoproteins identified until now in humans. The exact role is not known for about half of the selenoproteins. The amount of selenium found in blood and tissue is related to dietary intake [5, 6, 7].

2. Biological activity

The biological role of selenium was unknown until the middle of the twentieth century, when Schwarz and Foltz [6] showed that necrotic liver degeneration can be caused by selenium deficiency. Later, in 1973 selenium was found in the composition of mammalian enzymes, such as glutathione peroxidase [7]. In the intervening years, the essential characteristic of selenium for human nutrition was established.

Regarding the biological role of selenium, one can mention the fact that it is part of the endogenous antioxidant defence system. The function of this system is to protect cells against the attack of reactive oxygen species [8]. Selenium also plays a role in normal spermatogenesis, the maintenance of normal hair and nails, normal function of the immune system and normal thyroid function [9, 10].

Selenium is found in the structure of the enzyme glutathione peroxidase (that acts as a scavenger of hydroperoxides, phospholipid hydroxiperoxides), selenoproteins, some amino acids and 5'-deiodinase (involved in thyroid metabolism). The absorption of selenomethionine takes place in the small intestine by a carrier-mediated process.

Absorption of selenium can be studied by using the activity of the enzyme glutathione peroxidise since this enzyme contains selenium-amino acid residues. Using this method, the relative bioavailability of selenium from different food sources can be evaluated. Labelled selenium (⁷⁵Se) can also be used in the study of absorption.

Plasma biomarkers like selenium concentration and selenoprotein concentration were also used to evaluate the bioavailability of various forms of selenium. The plasma selenium concentration was raised in a dose-dependent manner by L-selenomethionine and high-selenium-enriched yeast.

After absorption, L-selenomethionine is metabolised to hydrogen selenide (H_2Se) via selenocysteine or methylselenol. This process can be followed by conversion to selenophoshate (HSePO₃), which is then incorporated into essential selenoproteins. The surplus of hydrogen selenide is further metabolised. The resulting compounds, methylated derivatives or

selenosugars are excreted in urine. They can also be oxidised to selenium dioxide, a pathway associated with toxicity due to the production of reactive oxygen species.

Selenium from selenite - following oral intake and absorption - is found in the highest concentrations in the liver and kidneys. After absorption, selenium is converted to hydrogen selenide (H_2Se). From this point on, the same reactions take place as in the case of L-selenomethionine [11].

3. Distribution in foods and requirement

Given the importance of selenium for the normal function of the human body various sources have been devised of assuring its optimum intake.

3.1. Vegetal and animal sources

The main vegetal sources of selenium are cereals and vegetables. The amount of Se in such products varies conditioned by the soil content in selenium, therefore by the geochemical characteristics. Depending on their ability to assimilate and accumulate, plants can be classified into "selenium accumulators", e.g. rapeseed, broccoli, cabbage, garlic, onions, leeks and "non-selenium accumulators", e.g. wheat, oats, rye, barley (Table 1).

Specification	Content (mcg / 100 g)	Specification	Content (mcg / 100 g)
Brazil nuts	230	Pistachio	6,8
Sesame seeds	56	Graham bread	3,1
Peanuts	30	Peanut butter	3,0
Cashew nuts	15	Barley	2,8
White rice	13	Almonds	2,2

 Table 1. Selenium content in plant foods (mcg per 100 g edible parts)

Food products of animal origin containing higher amounts of selenium are meat and viscera (liver, kidneys), fish, eggs, dairy products, etc. (Table 2). The amount of selenium in these products is closely related to animal feed.

Table 2. Seleman content in 1000 of animal origin (meg per 100 g)						
Specification	Content (mcg / 100 g)	Specification	Content (mcg / 100 g)			
Chicken	28	Salami	13			
Porc	17	Pike	24			
Beef	9	Egg yolk	59			
Lamb	7	Egg white	8			
Raw ham	15	Whole milk UHT	1,1			

Table 2. Selenium content in food of animal origin (mcg per 100 g)

3.2. Nutritional requirements

Expressed in mcg / day, it differs depending on age, physiological condition and are: for children: aged 7-11 months 15; 1-3 yrs 15; 4-6 yrs 20; 7-10 yrs 35; 11-14 yrs 55; 15-17 yrs 70 and for adults: \geq 18 yrs 70; Pregnant females - 70; Lactating females - 85.

Selenium requirements in humans is assured mainly by various foods of vegetal and animal origin. An other convenient way of ensuring an optimum selenium intake is represented by fortified foods or food supplements (concentrated sources of nutrients).

4. Food supplements with selenium

Currently, in the European Union, six forms of selenium can be used, i.e. selenium enriched yeast, selenomethionine and selenious acid, sodium selenate, sodium hydrogen, selenite sodium selenite while in fortified foods only four forms are admitted [12].

The evaluation of the various chemical forms of selenium was carried out by the European Food Safety Authority (EFSA), prior to their inclusion in the lists of admitted chemical forms.

a) Chemical compounds accepted in food supplements

In food supplements L-selenomethionine and selenious acid are the mostly used chemical forms. L-selenomethionine is a selenium containing amino acid in which selenium replaces the sulphur atom found in methionine [13].

The bioavailability of selenium from L-selenomethionine was shown to be higher than that from inorganic compounds of selenium. It is estimated to be greater than 90%.

Regarding the bioavailability of selenious acid, it can be considered equivalent to that of sodium selenite, since both of them dissociate to their component ions in the gastrointestinal tract.

b) Nutritional Reference Value

In the EU Regulation no. 1169 / 2011 - Annex XIII - the amount of 55 mcg per day was established as the Nutritional Reference Value (NRV) for selenium. A physiological effect is considered to be achieved when Se content is at least 15% of NRV.

5. Deficiency and excess

Deficiency of Se was observed in patients with selenium-free parenteral nutriton. In their cases symptoms of skeletal myopathy, muscle weakness a.o. were detected. A diet low in iodine and selenium is thought to be a risk factor for myxomatosis, too. It has also been found that selenium deficiency may be involved in organ and tissue degeneration with the onset of Keshan disease (endemic cardiomyopathy) and / or Kashin-Beck disease (chronic degenerative osteochondropathy).

Excess of Se - when the intake exceeds 1000 mcg/day - can lead to the appearance of selenosis. It is manifested by headache, thinning hair, nail deformation, rash, large number of tooth decay with discoloration of the teeth, paresthesias, paralysis and hemiplegia. In high doses, selenium itself has been shown to be carcinogenic.

Concluding remarks

Selenium is a trace element that can be found in lithosphere and hydrosphere and is an essential nutrient for humans in small amounts. It is present in various quantities in foodstuffs of vegetal and animal origin. In food supplements six selenium compounds are admitted as ingredients. Also, a number of four selenium compounds can be used to fortify various foods.

The current paper reviews information regarding the inorganic and organic compounds of selenium used in food supplements. It is also discussed the biological activity of selenium, its nutritional reference value in humans, its bioavailability, effects of Se deficiency and excess.

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ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM BLACK ELDERBERRY FRUIT

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Abstract

The objective of this study was to determine the most adequate process parameters, using a green extraction method, which ensures maximal utilization of *Sambucus nigra* L. fruit. Ultrasound-assisted extraction was applied for the isolation of phenolic compounds from elderberries and for obtaining extracts rich in anthocyanins. The UAE experiment was performed at sonication amplitude in the range from 20 to 100%. The highest extraction yield (47.28%) was achieved at 100% amplitude, while the lowest yield (27.70%) was achieved at 20% amplitude. The UAE extract of elderberry obtained at amplitude of 100% proved to be the best in terms of the content of total phenols and monomeric anthocyanins. According to the obtained results, it can be concluded that the suggested extraction approach has a potential for the production of new pharmacologically-active fractions.

Introduction

Sambucus nigra L. belongs to the Adoxaceae family and naturally occurs in most of Europe. Flowers and fruit are rich in dietary phytochemicals, such as carbohydrates, proteins, lipids, fatty acids, phenolic acids, flavonoids, organic acids, minerals, vitamins, etc., that give them a high commercial value [1]. In Europe, elderberries are used for centuries in food industry to produce pies, jams, jellies, ice creams, and yogurts [2]. Polyphenols are the most important group of bioactive compounds present in elderberry in relatively high concentrations. Anthocyanins, as well as other flavonoids, exhibit antioxidant, anticarcinogenic, immunestimulating, antibacterial, antiallergic, and antiviral properties. Their consumption may contribute to prevention of several degenerative diseases such as cardiovascular disease, cancer, inflammatory disease, and diabetes [3].

Experimental

Ultrasound-assisted extraction (UAE) was performed in an ultrasound probe (Hielscher Ultrasonic GmbH, Germany), which operated in continuous mode at a constant extraction time of 6 min. The milled elderberry fruit was added to an ethanol-water solution (30%) and the effect of sonication amplitude in the range from 20 to 100% was evaluated. According to our preliminary results, the selected extraction solvent showed the best extraction properties for this raw material. During the process, an increase in extraction temperature was measured. In obtained extracts, the content of total phenols and the content of monomeric anthocyanins were determined using spectrophotometric methods.

Results and discussion

The highest extraction yield (47.28%) was achieved by using 30% ethanol during 6 min, at 100% amplitude, while the lowest yield (27.70%) was achieved at 20% amplitude. The change of the content of total phenols and anthocyanins followed the same trend as in the case of extraction yield. The UAE extract of elderberry obtained at amplitude of 100% proved to be the best in terms of the content of the investigated compounds. The highest total phenolic

content in elderberry extract was 4.23 mg GAE/mL of extract, while the highest monomeric anthocyanin content was 24.36 mg/L of extract. Total phenolic contents in elderberry extracts obtained at amplitudes of 20 and 60% were 2.52 and 3.61 mg GAE/mL of extract, respectively. During the process, an increase in temperature was recorded by applying all three amplitudes. As expected, the highest increase was recorded at amplitude of 100%, which was 66 °C after 6 min, while a lower increase in temperature was measured at 20 and 60% amplitudes (33 °C and 55 °C, respectively).

Conclusion

Based on the obtained results, the UAE by ultrasonic probe proved to be suitable for isolation of phenolic compounds from elderberry fruit. The highest amplitude of ultrasound gave the best results in terms of the content of phenolic compounds, as well as anthocyanins, as the most important compounds in elderberry fruit due to their health-promoting effects. In future work, the influence of an increase in extraction time above 6 min on the extraction temperature will be investigated.

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EFFECT OF IRRADIATION WAVELENGTH ON THE HETEROGENEOUS PHOTOCATALYTIC REMOVAL OF ORGANIC POLLUTANTS USING TiO₂ AND ZnO

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Abstract

The efficiency of TiO₂ and ZnO was compared during the photocatalytic removal of neonicotinoid pesticides (imidacloprid and thiacloprid) and sulfonamide antibiotics (sulfamethazine and sulfamethoxypyridazine), causing severe environmental and health problems. The differences between LEDs emitting at 365 nm and 398 nm were compared, and removal efficiencies were tested in tap water and biologically treated wastewater matrices. The effect of the most abundant anions, Cl⁻ and HCO₃⁻ on the removal efficiency and 'OH formation was also compared. TiO₂ was more sensitive to the matrices and the irradiation wavelength. 'OH production was higher for TiO₂, and 398 nm photons resulted in a higher contribution of 'OH. Efficiencies were not reduced by matrix components for ZnO, which is mainly the result of increased 'OH-production by Cl⁻. In the case of TiO₂ and 365 nm photons, the formation of CO₃⁻⁻ from HCO₃⁻⁻ was assumed. For TiO₂, the significant inhibition of matrices could not be explained solely by the effect of anions.

Introduction

Pesticides and pharmaceuticals are amongst the most common organic pollutants found in wastewaters, and they have been detected in water bodies. Even in low concentration, they might have several chronic effects (e.g., carcinogenic, teratogenic, endocrine disruptor), and their accumulation in the ecosystem leads to further problems [1]. The removal of these trace amounts of pollutants is inefficient with the currently used traditional water treatment methods; therefore, the application of additive processes to be required.

Advanced Oxidation Processes (AOPs) may offer a way to remove trace amounts of pollutants using highly reactive radicals. Heterogeneous photocatalysis is a widely investigated AOP, based on the irradiation of a semiconductor. The absorption of photons with appropriate energy causes a charge separation and leads to electron-hole pair formation. These photogenerated charge carriers directly oxidize/reduce the pollutants or lead to reactive oxygen species (ROS) formation via further reactions. Generally, the most important ROS is the hydroxyl radical (OH) due to its high reactivity and low selectivity [2]. The most widespread photocatalysts are TiO₂ and ZnO, both having a relatively wide bandgap (3.0-3.3 eV); therefore, UV light is needed for their efficient excitation. Due to the drawbacks of traditional UV lamps, lightemitting diodes (LEDs) have recently gained popularity in water treatment. LEDs often offer longer lifetimes, higher electric efficiencies, lower prices, and more flexible photochemical designs [3]. The intensity and wavelength of the light source is probably the most important factor determining the amount and lifetime of electron-hole pairs, thus directly affecting ROS formation [4,5], but results - even for the most well-characterized photocatalysts - are few and sometimes contradicting. UV-LEDs may offer a simple way to investigate these effects thanks to their relatively narrow emission spectra and precisely tunable intensity. Beyond energy consumption, the other primary obstacle for the practical application of photocatalysis is the complex effect of matrices. The components of the treated water can lead to significantly

hindered photocatalytic activity, either by scavenging ROS/photogenerated charges, occupying the surface sites of the catalyst surface or by increasing the aggregation of the particles. The results must be carefully evaluated, as matrix components may even increase the efficiency in some cases due to the formation of selective radicals from inorganic ions (e.g., SO_4^{-} , CO_3^{-} , Cl[•]) [6]. The effect of matrices is relatively rarely investigated in detail, especially for photocatalysts other than TiO₂ [7,8].

This work aimed to compare the efficiency of two commercial photocatalysts, TiO_2 and ZnO, for the removal of four organic pollutants, two neonicotinoid pesticides, imidacloprid (IMIDA) and thiacloprid (THIA), and two sulfonamide antibiotics, sulfamethazine (SMT) and sulfamethoxypyridazine (SMP). Two UV-LEDs, emitting at 365 nm and 398 nm were used, and the effect of two water matrices, tap water and biologically treated wastewater (BTWW) were investigated. The effect of their most abundant inorganic components, CI^- and HCO_3^- were also compared. The 'OH formation rates were compared using coumarin (COU), as the formation rate of its hydroxylated product, 7-hydroxy-coumarin (7-HC) correlates well with the formation rate of 'OH.

Experimental

During the photocatalytic experiments, commercially available photocatalysts, TiO_2 Aeroxid[®] P25 (Acros Organics) and ZnO (Sigma Aldrich) were used, the concentration of their suspensions was 1.0 g dm⁻³. Two photoreactors were used, one was equipped with 12 high-intensity UV-LEDs emitting at 365 nm (Vishay, VLMU3510-365-130, 2 W/LED), the other with a UV-LED tape with 50 diodes emitting at 398 nm (LEDMaster, 0.09 W/LED). An AX-3005DBL-3 laboratory power supply was used to provide and control the electrical power needed to operate the light sources. In the case of 365 nm LEDs 200 cm³ suspensions were irradiated, and 100 cm³ in the case of 398 nm LEDs. The suspensions were constantly being bubbled with synthetic air during the measurements. Before the measurements, the suspensions were stirred in the dark for 20 minutes. Before analysis, samples were centrifuged at 15000 RPM and filtered using a 0.22 μ m PVDF syringe filter.

For the investigation of 'OH-formation, 5.0×10^{-4} M COU was used; its concentration was determined via UV-Vis spectrophotometry (Agilent 8453) at 277 nm. The formation of 7-HO-COU was measured using fluorescence spectroscopy (Hitachi F-4500). The concentration of the neonicotinoids and sulfonamides was determined via HPLC system (Agilent 1100). A Lichrosphere 100 RP-18 5 µm column was used to separate the neonicotinoids and their products (mobile phase: 40 : 60 MeOH : H₂O mixture; 1.0 cm³ min⁻¹ flow rate; 30 °C). Detection of IMIDA and THIA was performed at 270 nm and 242 nm, respectively. For separation of SMT and SMP and their products, 30 : 70 MeOH : formic acid (0.1 v/v%) mixture as the mobile phase was used. UV-detection was performed at 265 and 261 nm. Initial reaction rates (r₀) were determined from the linear part of the kinetic curves (up to 20 % transformation of the model compound).

Results and discussion

The 'OH formation rate was compared using TiO₂ and ZnO under 365 nm and 398 nm irradiation. The transformation rate of COU and the formation rate of 7-HC were determined. In the case of 365 nm irradiation and TiO₂ lower transformation rate $(3.37 \times 10^{-7} \text{ M s}^{-1})$ of COU was measured compared to ZnO ($4.68 \times 10^{-7} \text{ M s}^{-1}$), but the formation rate of 7-HC was 40% higher in the case of TiO₂, indicating a more significant contribution of 'OH. The differences were less pronounced using 398 nm LEDs, but TiO₂ still produced higher amounts of 'OH. The yield of 7-HC was higher than 365 nm irradiation, indicating a higher contribution of 'OH when lower energy photons are used.

ZnO was more effective in eliminating each target pollutants than TiO₂ (8-50% difference). SMP, TiO₂ photocatalyst, and 398 nm irradiation caused an extremely high transformation rate $(7.37 \times 10^{-7} \text{ M s}^{-1})$ compared to the other experiments with 398 nm LEDs (2.48-6.43×10⁻⁸ M s⁻¹). It might be caused by the fact, that in the case of 398 nm photons, only the rutile (band gap: 3.0 eV) phase of TiO₂ is excited, resulting in a different transformation mechanism. It was confirmed that, in 398 nm radiated TiO₂ suspension, the main product of SMP formed via SO₂ extrusion, which was not detected during the other experiments.



Figure 1. The effect of tap water and biologically treated domestic wastewater (BTWW) on the relative transformation rates (r_0 / r_0^{REF}) of the model compounds using 365 nm and 398 nm LEDs

Experiments were performed in tapwater and BTWW matrices to investigate their effect on efficiency. In the case of TiO_2 , both matrices caused an adverse effect; only minor inhibition was measured in the case of sulfonamides. ZnO was less sensitive to the matrices; even a slight increase in the transformation rates was observed in some cases. The formation rate of 7-HC also increased, especially when using 365 nm LEDs, which indicate an increased 'OH formation (by 210-240%), as opposed to TiO_2 , where it was significantly inhibited (by 80%), explaining the decreased efficiency. When using 398 nm LEDs, similar effects were determined in both matrices, and the efficiency was further reduced when using TiO_2 . The matrices completely inhibited the fast transformation of SMP via SO₂-extrusion, resulting in similar reaction rates measured for the other model compounds.

To further investigate the complex effect of matrices, the effect of the most abundant anions – Cl^{-} and HCO_{3}^{-} – having radical scavenging ability was investigated. Their concentration was set to that measured in BTWW (120 mg dm⁻³ Cl⁻ and 525 mg dm⁻³ HCO₃⁻). Both ions have been reported to react with 'OH and photogenerated holes, and are responsible for the reduced

transformation rates. At the same time, their reaction with electron-hole pair results in more selective, less reactive radicals (Cl[•], CO₃^{•-}). Despite its high 'OH-scavenging capacity, in the case of TiO₂ Cl⁻ did not have a significant impact on the transformation rates, which may be the result of the reformation of 'OH from Cl[•] at neutral pH. In the case of ZnO, an enhanced transformation rate was detected for some model compounds, which could result from either hindered electron-hole recombination by adsorbed Cl⁻, or the formation of reactive chlorine species (RCS), which react more selectively with organic pollutants. Based on these results, we can conclude that, the positive effect of Cl⁻ on ZnO is caused by the reduced recombination of charge carriers and enhanced 'OH formation.



Figure 2. The effect of Cl^{-} and HCO^{3-} on the relative transformation rates (r_0 / r_0^{REF}) of the model compounds using 365 nm and 398 nm LEDs

 HCO_3^- did not affect or slightly lowered the transformation rates of the target compounds. Using 365 nm LEDs, the formation of 7-HC increased during the transformation of COU, which is the result of enhanced hydroxylation by CO_3^- . A similar effect was not detected in the case of ZnO, where CO_3^- does not form, either when using 398 nm irradiation with TiO₂. As opposed to Cl⁻, HCO_3^- completely inhibited the fast transformation of SMP, similarly to tapwater and BTWW. The effect of these anions seems to depend on the excitation wavelength, with a less pronounced positive effect and similar or higher inhibition in the case of 398 nm photons. But even their combined effect could not explain the loss of efficiency when using TiO₂ in water matrices. The significant difference may come from the mixed-phase of TiO₂ as only exciting the rutile phase by 398 nm photons might lead to different reaction mechanisms.

Conclusions

In the case of TiO_2 , the role of 'OH is more significant than ZnO, and a higher contribution of •OH was measured when lower energy photons were used. TiO_2 is more sensitive to the effect of matrices compared to ZnO. For TiO_2 , the effect of the irradiation wavelength is more significant due to its mixed phase with different bandgap energies. In the case of TiO_2 and 365 nm irradiation, less reactive CO_3^{-} forms via hole scavenging, reducing efficiency, and making the COU method unsuitable for 'OH-detection in the presence of carbonates. Cl⁻ has a positive effect when using ZnO, resulting in slightly increased efficiency in matrices due to increased 'OH-formation.

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CHANGES IN ANTIOXIDANT PROPERTIES OF FRUIT AND VEGETABLE CONCENTRATES UNDER THE EFFECT OF VACUUM DRYING

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Abstract

During our experiment, we examined changes in the antioxidant capacity and total polyphenol content of 2 types of 65% vegetable juices (carrots, pumpkin) rich in polyphenols, minerals, and retinoids, as well as two kinds of fruit juice concentrates (lemons, oranges) rich in citrus flavonoids. In our studies, the antioxidant/reducing properties of the preparations were measured and compared using TEAC and FRAP methods.

Our various analytical measurements found significant differences between the antioxidant capacity values and total polyphenol content of each fruit-vegetable juice concentrate and the same instant powders prepared from them before and after vacuum drying.

Introduction

By regularly consuming vegetables and fruits rich in vitamins, minerals, polyphenols and antioxidants, the risk of developing civilisation diseases with the highest mortality rates can be significantly reduced [1,2,3,4.] as free radical reactions can be delayed or inhibited by antioxidants.

However, one of the biggest obstacles to this is that the population's consumption of vegetables and fruits is mostly non-regular, only occasional. This can be due to several everyday difficulties (e.g., regular purchase, storage, the time-consuming process of fresh fruit and vegetables, the lack of controlled quality fruits free of chemical residues, etc.). An excellent alternative to them is the regular consumption of instant powders from controlled cultivations produced by vacuum drying from fruit and vegetable concentrates while maintaining their beneficial organoleptic properties, retaining their high nutritional values for a long time so that their regular consumption can contribute to maintaining health.

Materials and methods

From the 65% vegetable juice and juice concentrates, GPS Powder Ltd. produced the fruit and vegetable instant powders with gentle vacuum drying. The samples used for our study were obtained from GPS Powder Ltd.

Fruit and vegetable instant powders were produced in the tray LMIM LP-405 vacuum oven with four parallel trays drying (by sensing temperature per tray), in a pressure range from 20 mbar to atmospheric pressure, at a temperature between 10°C and 40°C, with a short heating time of 240 minutes. The resulting solid was pulverised in a grinder and



stored in a sealed polyethylene bag at a temperature below 20°C until measurements began. For analytical measurements, the 65% water concentrates were tested independently and prepared, if necessary, diluted with distilled water. We made an aqueous solution from the vacuum-dried powders, which was placed in a cooled ultrasonic water bath for 30 minutes, and then the samples were centrifuged at 13500 rpm. In all cases, the pure supernatant was used for the test.

Analytical methods

Determination of antioxidant capacities by TEAC (Trolox-equivalent antioxidant capacity) method: The total antioxidant capacity was measured with the Trolox-equivalent antioxidant capacity (TEAC) method described by Miller et al. (1993) [5.]. The method is based on ABTS+ free radical scavenging by antioxidants measured with a spectrophotometer. For the calibration Trolox (the hydrophilic analogue of vitamin E) was used.

Determination of antioxidant capacities by FRAP (Ferric Reducing Antioxidant Power) method: Measurement of ferric reducing antioxidant power of the peel extracts was carried out based on Benzie and Strain's procedure [6.], at 593 nm. Ascorbic acid (AA) was used as a standard to prepare the calibration solutions. Results were expressed as µMAA/g DM.

Determination of total phenolic contents (TPC) by Folin-Ciocalteu method: The Folin-Ciocalteu spectrophotometric method by Singleton and Rossi [7], at 760 nm is an electron transfer based on assay and shows the reducing capacity, which is expressed as phenolic content. Gallic acid (GA) was used to prepare the standard curve. The results were expressed as μ M GA/g of dry matter (DM).

Results and discussion

I.) Total phenolic contents (TPC) measurement results

The analysis of the total polyphenol content of the four types of water concentrates and the instant powders, obtained from them by gentle vacuum drying, showed that *vacuum drying* resulted in a *significant increase* in the *total polyphenol content* of vegetable powders (carrot powder: 33.22%, pumpkin powder 43.09%), while *citrus fruit* powders *decreased significantly* (lemon powder 60.13%, orange powder 76.7%) (Fig.1).



Figure 1. Total polyphenol content (uMGS/g) of fruit and vegetable juice concentrates and the differences between their instant powder variants.

II.) Measurement results of antioxidant capacity by TEAC and FRAP methods

Antioxidant capacity can be defined as the combined effect of all antioxidant compounds found in a system, for which more than one hundred methods have been developed [8]. All test methods have their advantages and disadvantages. Still, we cannot correctly model the biochemical processes in the body with any of them, so it is of the utmost importance to draw conclusions about the sample examined not only based on one but several methods of analysis. Our test results showed that in the study of antioxidant capacity by the TEAC and FRAP methods of 65% juice concentrates, a significantly higher value could be measured in all juice concentrate samples using the TEAC method than with the FRAP method (Fig.2). Particularly significant for pumpkin juice, where the difference is 91.26% (Fig.2).

On the opposite, for instant powders made by vacuum drying, the antioxidant capacity values measured by the FRAP method were higher than by TEAC method. Only oranges are the exception, for which the difference is only 10.46% (Fig.3).

Comparing vegetable juice concentrates with fruit juice concentrates, we found that the TEAC method measured a higher antioxidant capacity in vegetable juice concentrates than fruit juice concentrates rich in citrus flavonoids. The highest antioxidant capacity was measured in the pumpkin (30.08), followed by carrot juice (26.37), followed by orange juice (24.72) and lemon juice (15,21 uMtrolox/g) with a more significant difference (Fig.2.).

At the same time, we saw the opposite results with the FRAP study: in fruit juice concentrates, the FRAP method measured higher antioxidant capacity values than vegetable juice concentrates: significantly the lowest value for pumpkin juice (2.62 uMAS/g). Lemon juice (11,76) and carrot juice (11,10) showed nearly identical values, with the highest FRAP measured for orange juice concentrate (14,72uMAS/g) (Fig.2).



Figure 2. Antioxidant capacity of fruit and vegetable juice concentrates (j. cc.) by TEAC and FRAP measurement methods



Figure 3. Antioxidant capacity of fruit and vegetable instant powders by TEAC and FRAP measurement methods.

Vacuum drying resulted in a significant reduction in antioxidant capacity in instant fruit and vegetable powders compared to the juices using both TEAC and FRAP methods, except in the case of pumpkin powder, where the antioxidant capacity measured by the FRAP method increased by 61.12 % (from 2,6 to 6,7 uMAS/g) (Fig.4-5).



Figure 4. Changes in the antioxidant capacities of the concentrates and instant powders made from them by vacuum drying and their differences in % by FRAP method (uMAS/g)
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Figure 5. Changes in the antioxidant capacities of the concentrates and instant powders made from them by vacuum drying and their differences in % by TEAC method (uMTrolox/g).

Conclusion

Considering the wide-ranging and very beneficial physiological effects of polyphenols in our body, significant changes in all polyphenol content due to vacuum drying (increase in carrots powder and pumpkin powder and decrease in lemon powder and orange powder) can be important clues for food manufacturers and product developers in the development, production and use of each combined final product.

The importance of appropriately choosing antioxidant capacity testing methods is highlighted by the fact that in our various analysis studies (FRAP and TEAC), we found significant differences between the antioxidant capacity values of the same vegetable juice and fruit concentrates and the same instant powders.

Knowledge of the changes in total polyphenol content and the significant differences in antioxidant capacity under the effect of vacuum drying could determine which fruits and vegetables are the best suited for vacuum drying technology to preserve their content values.

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THE ROLE OF TEMPERATURE IN THE BIOACCUMULATION PROCESSES OF MANGAN FROM SURFACE WATER, IN SPECIES SALVINIA NATANS (L.) ALL.

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Abstract

In this article we wanted to demonstrate the influence that temperature has on the processes of manganese accumulation in the species *Salvinia natans* (L.) All. In order to reach the proposed objective, different concentrations of manganese present in the water were tested at different temperature levels (15° , 25° and 35° C) and the samples of plant material were analyzed at different time intervals (0h, 24h, 72h and 120h).

Introduction

Water supply is the key to civilization and offers development opportunities in a certain geographical area. Assessing the water supply for domestic and industrial use requires a broad approach to define and determine the socio-cultural, demographic and economic benefits of water. A number of studies highlight the important role of water availability in maintaining and advancing living standards [1,2]. Water resources are open to pollution due to population growth, technological development and growth of industrial activity. In many regions of the world, in urban areas, the metal load in drinking water resources is at an alarming level, due to the elimination of untreated or partially treated industrial wastewater [3,4]. Because heavy metals are difficult to neutralize under natural conditions, they are usually ingested by aquatic animals and plants, as well as by terrestrial crops and then enter the human body through the food chain after a high enrichment in the propagation bodies [5].

Salvinia natans (L.) All. is a fast-growing aquatic pteridophyte of the Salviniaceae family, native to large regions of Asia, Europe and Africa and introduced to North America. The species produces creeping roots up to 20 cm long, with up to 12 axillary branches, and two types of leaves, floating and submerged. The floating leaves are 0.8–1.4 cm long and 0.5–0.8 cm wide. The submerged leaves are dissected into linear segments, covered with hair and can act as roots. The typical habitats of S. natans are ponds and ditches with slow and shallow eutrophic water [6]. Salvinia sp. it can double its biomass in less than 2 days. Moreover, the plant can have a high productivity around 5.8-11.4 g in dry weight/ m^2 /day when grown in a chemically defined Hoagland environment and around 20-120 kg/ha/day under natural conditions [7], these things are especially important in order to be able to ensure the vegetal material and to make efficient the purification systems based on this species. In addition to its high growth, the species accumulates trace elements and has been used to remove both nutrients and metals from polluted waters [6]. Dhir and Srivastava (2011) demonstrated a gradual decrease in metal content in wastewater samples with an initial metal content of 15 mg Me/L by renewing plant biomass at well-defined time intervals. The elimination of Zn, Cu, Ni and Cr in the proportion of 84.8%, 73.8%, 56.8% and 41.4%, respectively, was noticed after four such plant renewals. According to the same authors, the accumulation of Cr, Fe, Ni, Cu, Pb and Cd was between 6 and 9 mg/g dry matter, while the accumulation of Co, Zn and Mn was around 4 mg/g dry matter [8]. Based on these considerations, this article aims to streamline the treatment of surface water loaded with manganese in specific laboratory conditions and at different temperature levels, using the species *Salvinia natans* (L.) All.

Experimental

Plant material

The experiments were performed on plant material (*Salvinia natans* (L.) All.) harvested from the natural environment and acclimatized in laboratory conditions for seven days. The test solutions in which the plants were introduced were manganese-enriched surface waters (surface waters had an imperceptible load of heavy metals) to reach the desired concentrations. The water needed for the experiments was taken from the river Bega, shortly before the start of the study. The reagents used for water enrichment were stock, monometallic solutions with a concentration of 1000 mg Me/L purchased from specialized companies in the field.

Experimental conditions

Approximately 10 grams of plant material, representing 6 individuals with 5-6 nodules each, were placed in transparent plastic containers with a diameter of 8 cm containing 300 mL of surface water enriched up to concentrations of 1, 2 and 4 mg Mn/L. The amount of metal was determined both initially (0h) and after 24h, 72h and 120h experimentally during which the plants stayed in the incubator at different temperature ranges (15° C, 25° C, 35° C \pm 0.5° C) having a day/night cycle of 16/8. The choice of metal concentrations was consistent with the 5th grade of quality from the Romanian Order nr. 161/2006 [9], which reports values for Manganese >1000 µg/L, and by doubling them twice consecutively, the above concentrations were reached.

Methods of analysis

After various drying, calcination and digestion processes, the plant material was brought to liquid state, and the determination of the metals in the samples was performed with an Avanta GBC AAS spectrophotometer (GBC Scientific Equipment Pty Ltd, Australia) in accordance with existing standards.

Bioaccumulation factor (BCF)

The accumulation of a chemical by aquatic organisms is generally expressed as a bioaccumulation factor (BCF). This factor is expressed as the ratio between the final concentration of metal ions in the plant and the initial concentration of metal in water. It is an indicator of the accumulation capacity of metals by plants at the metal concentration in the environment [10].

$$BCF = \frac{Cme-plants}{Cme-water}$$

where,

 $C_{me-plants}$ = final metal concentration in the plant, expressed in mg Me/kg C_{me-apa} = initial concentration of metal in water, expressed in mg Me/L

Results and discussion

The table below shows the amount of manganese accumulated in the tissues, at each temperature level and after each time interval.

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Stress	The amount of Mn accumulated in Salvinia natans, mg Mn/kg s.u.							g s.u.				
factor	15°C			25°C				35°C				
	0h	24h	72h	120h	Oh	24h	72h	120h	0h	24h	72h	120h
1 mg	82.0	175	386	504	82.0	113	315	262	82.0	87.0	530	614
Mn/L												
2 mg	82.0	553	1039	805	82.0	361	490	798	82.0	256	495	591
Mn/L												
4 mg	82.0	540	1024	993	82.0	125	664	337	82.0	90.0	97.0	144
Mn/L												

Table 1. The amount of manganese accumulated by *Salvinia natans* under different temperature conditions and after certain periods of time

The results obtained demonstrate the ability of this species to accumulate manganese, specific for each temperature range and for the initial concentration of metal in the environment. The data obtained by us are in accordance with those in the literature, where Dhir and Srivastava (2011), report values of manganese in the plant located around 4000 mg/kg s.u., at an initial metal concentration of 15 mg Mn/L [8]. Therefore, the present species is very suitable for bioremediation of manganese-laden surface waters, and to determine the ability of the species to accumulate metals compared to the amount of pollutant found in the environment, the values calculated for the bioaccumulation factor are presented below.

Bioaccumulation factor (BCF) of Mn in Salvinia natans (L.) All.



Figure 1. Mn bioaccumulation factor for *Salvinia natans* at 15° C

At a temperature of 15°C, we observe an affinity of the plant for the bioaccumulation of manganese at low concentrations. Here we observe the highest values of BCF in the case of concentrations of 1 and 2 mg Mn/L. The hypothesis is also supported by the fact that we find the highest value (520) of the bioaccumulation factor in the case of the experimental variant with 2 mg Mn/L, after the first 72 hours.

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Figure 2. Mn bioaccumulation factor for Salvinia natans at 25° C

At a temperature of 25 ° C, the bioaccumulation processes in *Salvinia natans* gradually increase in the first 72 hours for the initial metal concentrations of 1 and 4 mg Mn/L, after which it decreases in the next 120 hours. In the case of the experimental variant with 2 mg Mn/L, the increase of the bioaccumulation factor is directly proportional to the contact time, so that a maximum of 399 is reached after 120h. As at the previous temperature, the ability of the species to accumulate metals depends on their concentration in the environment, so the most satisfactory results are obtained at a concentration of 1 and 2 mg Mn/L in the environment.



Figure 3. Mn bioaccumulation factor for Salvinia natans at 35° C

At a temperature of 35° C, the highest bioaccumulation factor of 614, is obtained after 120h in the case of the 1 mg Mn/L variant. There is a gradual decrease of the bioaccumulation factor from a maximum of 614 in the case of a concentration of 1 mg Mn/L, to 296 in the case of a concentration of 2 mg Mn/L, to a minimum of 36 in the case of a concentration of 4 mg Mn/L. In an overview of the situation, we observe a drastic decrease of the bioaccumulation processes with the increase of the manganese dosage from the environment, similar to what was obtained at the other two temperature levels.

Conclusions

The present study demonstrated the vital role played by ambient temperature in the processes of bioaccumulation of manganese from surface waters by *Salvinia natans*, so that they are inversely proportional to the gradual increase in temperature (15° C > 25° C > 35° C). Regarding the concentration of metal in the environment, it is not recommended to use the plant at concentrations exceeding 2 mg Mn/L. The ideal exposure time is between 72h and 120h experimental, after which, if necessary, the plants can be renewed. Characteristics such as the

high rate of manganese accumulation and the short exposure time, make the species *Salvinia natans* suitable in phytoremediation studies.

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THE INFLUENCE OF DIFFERENT CORROSION SOLUTIONS ON THE TiO₂ MORPHOLOGY OBTAINED BY THERMAL OXIDATION OF TI FOILS

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Abstract

Titanium dioxide (TiO₂) is a well-known material having increased interest over the past decade in a wide range of application due to its advantageous features, such as long lifetime of excited electrons, excellent long-term chemical stability, non-toxicity, environmental safety, and relative low cost. Because of its large band gap (~3 eV) TiO₂ can absorb only in the UV region of the spectrum, this enabling it to be use in a wide range of applications like photo catalysis, UV and gas sensing and photovoltaic devices. The increasing of the surface/volume ratio of the nanostructures can also improve the targeted performance. The focus of this work is to show the influence of the chemical corrosion process, in acids and bases, on the surface morphology of the TiO₂ structure grown by the thermal oxidation of Ti foils. The synthesis of TiO₂ by corrosion growth and thermal oxidation is a low-cost process that can have a wide range of application. In this study, an aqueous mixture of HF was used as an acid corrosive solution, and as base solution NaOH aqueous mixture was used, both having the role of increasing the surface area of the titanium foils. After the chemical corrosion process, the titanium plate was washed with distilled water, then dried in vacuum condition. For the achievement of titanium oxide with high surface area structure, a thermal oxidation process has been applied in a controlled atmosphere consisting in a mixed gas flow of Ar and O₂, at a temperature of 500 °C for 4 hours. Moreover, by immersing titanium plates in an acidic solution followed by basic solution, and after a thermal oxidation process was applied, interconnected wires of TiO₂ layer was obtained. Scanning electron microscopy (SEM), X-ray diffraction (XRD), and UV-Vis spectroscopy were used to characterize the obtained TiO₂ nanostructure.



SEM images of the samples treated in (a) 0.05 M HF at a 48 h imersion time, (b) 5 M NaOH at a 48 h imersion time, and (c) 0.05 M HF at a 48 h imersion time + 5 M NaOH at a 48 h imersion time.

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COMPARATIVE MORPHOSTRUCTURAL AND ELECTROCHEMICAL CHARACTERIZATION OF POROUS Ti/SnO₂ CORRELATED WITH THE SYNTHESIS METHOD

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Abstract

In the recent years, one of the most important reasons for water pollution is the massive discharge of organics pollutants in water, which is characterized by high organic load and complex characteristics [1]. Electrochemical advanced oxidation processes (EAOP) for the wastewater treatment is attractive due to its simplicity, low energy consumption and environmental friendliness, but the central element is represented by the electrode correlated with the electrochemical reactor configuration. The promising electrode must fulfill four requirements including long lifetime, high catalytic activity, low cost and no secondary pollution. Much attention has been focused on dimensional stable anodes (DSAs) due to the advantages of high electric catalytic activity, low cost, simple preparation method [2]. DSAs exhibit high electroactive areas due to their mud-cracked morphology, alowing the direct oxidation of organic pollutants on the electrode surface at low potentials and are able to promote the formation of active intermediates (·OH, Cl⁻) to perform the indirect oxidation of pollutants [3]. DSAs electrodes consist of a titanium substrate covered with an oxide coating, whose composition strongly affects their electrochemical behavior. Considering the main mechanistic aspects related to the reactant and product transport to and from the electrode surface and the electron transfer, as stages of the overall electrode process, so-called three-dimensional stable anodes (3-DSAs) characterized by large porosity should exhibit enhanced electrooxidation activity towards the organic pollutants through improvement of the transport stage rate.

This paper presents a comparative study of two methods, *Dr. Blade* and *spin-coating* protocols, for synthesis of porous Ti/SnO₂ dimensionally stable anode suitable for advanced treatment of water/wastewater. The morphological differences of SnO₂ films appeared because of the type of SnO₂ specific to each synthesis method. Thus, it was used a cristalline powder into polimer matrix of SnO₂ for *Dr. Blade* method, and the amorphous gel of SnO₂ for *spin – coating* method. The morpho-structural characterization through X-ray diffraction (XRD) and scanning electron microscope coupled with energy-dispersive X-ray (SEM/EDX) confirmed a uniform and compact deposition of SnO₂ on Ti surface with typical "mud-cracked" like structure, for both synthesis methods. However, more porous structure of SnO₂ was noticed for Dr.Blade method in comparison with spin-coating that led to a more compact film of SnO₂. Electrochemical behaviours of both electrodes were studied by cyclic voltammetry in 0.1 M Na₂SO₄ and respective, in 0.05 M Na₂SO₄ and 0.05 M NaCl and in the presence of 5 mg·L⁻¹ Doxorubicine (DRC), a cytostatic from emerging pollutants class from water.



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THE EFFECT OF THE PRESENCE OF GOLD NANOPARTICLES ON THE LASER INDUCED BREAKDOWN IN ARGON GAS

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Abstract

Noble metal nanoparticles can greatly affect the sensitivity and selectivity of many spectroscopic techniques, thus they are widely used in the analytical chemistry. In this current study we investigated the effects of the presence of gold nanoparticles on the formation of laser-induced breakdown plasmas in argon gas.

Introduction

Laser induced breakdown spectroscopy (LIBS) is a laser based elemental analytical technique, capable to deal with solid, liquid and gaseous samples as well with very little sample preparation [1,2]. However, gaseous samples require much higher pulse energy compared to the case of solid samples, thus the cost of instruments capable of measuring such samples is significantly higher, and at the same time the detection limits for gases are inferior [3].

Nanoparticle-enhanced laser-induced breakdown spectroscopy (NE-LIBS), first described by de-Giacomo et al., is becoming a widely tested signal enhancing technique in recent years [1]. Properly sized and distributed metallic nanoparticles (NPs) deposited on the surface of a solid sample under some conditions proved to provide multiple orders of signal enhancement. The effect is generally described as being essentially caused by electron field emission. This technique proved to be efficient to increase the signal not just for solid, but also for liquid samples, however gas samples, which generally have a 10-100 times higher breakdown thresholds than solids and liquids, have not yet been attempted to be analyzed by the NE-LIBS approach.

Spark discharge nanoparticle generation is a promising physical technique for nanoparticle manufacturing. In spark discharge generators (SDGs), high-voltage and high-current, microsecond-long spark discharges are created between two electrodes in a controlled gas flow at atmospheric pressure. Due to the sparking, the electrode material is eroded and a vapor plume is formed between the electrodes, which then undergoes nucleation, condensation, coagulation, and aggregation [4].

In our study we used spark discharge generated nanoparticles to study whether the presence of nanoparticles in the gas medium is able to significantly lower the plasma formation threshold power density for the gas medium. Correlations between the magnitude of the effect and the size and number concentration of the nanoaerosol were investigated.

Experimental

The central part of the spark discharge generator system used here for the generation of gold nanoparticles is a vacuum chamber, in which the applied cylindrical, gold electrode pair was horizontally positioned and axially aligned with a 2.0 mm gap left between them (spark gap). The 5.0 standard liter per minute (slm) argon (99.996% purity) carrier gas flow, controlled by a mass flow controller was fed in the chamber via the bottom port of the chamber (upward pointing "crossflow" with injector nozzle) [5]. All experiments were carried out at near atmospheric pressure. A 8nF, monolithic, high voltage, pulse discharge capacitor (Model 450PM980, General Atomics) was connected to the spark gap and charged by a high voltage capacitor charging power supply (Model HCK 800-12500, FuG GmbH). The discharge of the capacitor between the electrodes commences when the voltage on the capacitor reaches the breakdown voltage in the electrode gap. The resulting spark discharge is a bipolar, oscillatory discharge. The repetition rate of the sparking, which affects the size distribution and number concentration of the generated aerosol, can be adjusted by controlling the charging current of the capacitor. The created gold NPs were lead through a 900 °C compaction furnace, so the attached primer particles are melted into solid particles. The concentration of the particles was controlled by a conventional (VKL 10, Palas GmbH) and/or a self-built dilution systems.



Figure 1. Schematic of the experimental setup

The LIBS experiments were carried out in a flow-through, small volume measurement chamber constructed in-house for LIBS aerosol measurements [6]. The Nd:YAG laser emitted of 10 ns long pulses at the fundamental 1064 nm wavelength, while the pulse energy was changed between 30 and 60 mJ. The beam was focused into the chamber from above through a UV-grade fused silica window. The repetition rate of the plasma generating laser was set to a low value (ca. 1 Hz) thereby ensuring that the content of the chamber is completely renewed by the gas flow between laser shots. The LIBS plasma was observed via two fused silica collimating lenses implemented in two ports located on the sides of the chamber (looking onto the same spot in the chamber in the horizontal direction, with 90° angle between the optical axes of the

two lenses). The collected light was coupled into a two-channel fiber-optic CCD spectrometer (AvaSpec-FT2048, Avantes, NL) using optical fibers. The measurement chamber was mounted on a translation stage, which allowed bringing the laser focal spot inside the chamber in front of the light collection lenses.

Gating of the spectral data collection was achieved by the internal electronics of the spectrometer, which was triggered by the laser power supply unit and continuously monitored on a digital storage oscilloscope (TDS1002, Tektronix, USA). The minimum possible gate delay of 1 μ s and gate width of 2 ms was set at the spectrometer. The double-channel spectrometer allowed the recording of the plasma emission in the 344–888 nm spectral ranges, with resolutions of 0.09 nm and 0.4 nm, respectively

Results and discussion

Our first exploratory results already proved the effect of nanoparticles, since their presence made it possible to generate laser induced plasma in the gas under conditions which resulted in no plasma when NPs were absent. Most of the intensive spectral lines observable in the LIBS sepctrum are argon atomic lines, while many of the less intensive lines were assigned to the Ar II species. However, none of the known strong gold spectral lines were noticeable in the spectra, which indicates that – according to the expectations - the small mass of gold in the blast radius must have been below the detection limit of our instrument. Although plasma formation was achieved at relatively low pulse energies already, but the plasma formation was found to have a random occurrence, thus the following data represents averaged data recorded for 100 laser pulses.



Figure 2. LIBS spectrum of clean argon gas and argon gas mixed with gold nanoparticles

Nanoaerosols with similar number concentration, but bigger mode of size distribution were found to produce microplasma at a similar frequency, but the average intensities were higher, meaning that the intensity of individual spectra increased. Using aerosols of the same size distribution, but with different number concentration, as well as in similar number concentration, but with different size distribution, the individual effect of the two aerosol parameters could be investigated. It was found that the increase in particle number concentration resulted in an increase of the average intensity on every pulse energy. The increased average intensity can be traced back to the increase of the plasma formation frequency and the higher intensities of the individual spectra as well. By looking at the Figure 3. the differences in the intensity curves are also visible. The increasing aerosol number concentration makes the slopes of the intensity curves not just steadier, but it shifts them towards lower pulse energies as well. This can be clearly interpreted as a correlation between plasma ablation threshold and aerosol number concentration.



Figure 3. Intensity of a selected argon atomic line as a function of laser pulse energy on different aerosol number concentrations

In our opinion, the random occurance of plasma formation and its increase by the aerosol number concentration can be explained by the random distribution of the nanoparticles in the carrier gas. The nanoparticles are much easier to break down compared to gas, thus if a nanoparticle is present within the focal spot (or its close vicinity) of the laser beam, then they can provide electrons for the ignition of gas plasma. When sufficient number of nanoparticles are near enough to the focal point of the laser beam during the laser impulse, then the microplasma is formed. Thus, the increase in aerosol number concentration increases the probability of particles being in this volume, thus an increase in the plasma formation can be observed. If the number of nanoparticles present in this volume exceeds the minimum necessary for plasma formation, then above this threshold the number of the ejected electrons will be proportional to the total particle volume (mass), thus also with the particle size and number concentration.

Conclusion

The possibility of the plasma formation threshold lowering using dispersed nanoparticles were presented. The signal enhancing effect was proven to related to both the number concentration and size distribution of the applied aerosol. We also pointed out the dependence of the threshold lowering on the aerosol number concentration. Finally, a possible explanation for this phenomenon was suggested as well.

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STRONTIUM TITANATE PHOTOCATALYSTS: PREPARATION, CHARACTERIZATION AND PHOTOCATALYTIC ACTIVITY

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Abstract

In this study strontium titanate photocatalysts were prepared *via* a hydrothermal method. The effect of various synthesis parameters (synthesis time, pH) on the properties of the samples obtained was investigated. The photocatalysts were characterized by X-ray diffractometry, scanning electron microscopy and transmission electron microscopy. The photocatalytic activity of the samples was evaluated by the photocatalytic reduction of carbon dioxide. A direct correlation was observed between the efficiency and strontium carbonate content of the photocatalysts.

Introduction

Due to the utilization of fossil fuels, carbon dioxide (CO₂) is constantly being emitted into the atmosphere. CO₂ is a well-known greenhouse gas that can trap heat, leading to global warming. Consequently, developing techniques that can reduce the concentration of CO₂ in the atmosphere is of utmost importance. Strontium titanate (SrTiO₃) is a promising candidate to address this issue. This material has beneficial properties such as long lifetime of electron–hole pairs, high chemical/thermal stability, and high catalytic activity [1]. To carry out photocatalytic reduction reactions, the energy of photoexcited electrons (and conduction band) must be higher than the redox potential of the reduction reaction of interest [2]. SrTiO₃ is considered to be a semiconductor with a wide band gap, and such materials are the most suitable for CO₂ photoreduction [3]. The reason for this is that they provide sufficiently negative and positive redox potentials concerning their conduction and valence bands, respectively. Taking these factors into account, in this work SrTiO₃ photocatalysts were prepared and their applicability for CO₂ photoreduction was investigated. Emphasis was put on examining the casual relationship between the synthesis parameters and efficiency.

Experimental

For the preparation of SrTiO₃ photocatalysts, multiple synthesis procedures were applied. Based on the publication of Ramos et al., 5 M NaOH (80 mL), 2 M urea (10 mL) and 3 M Sr(NO₃)₂ solutions were prepared [4]. After magnetic stirring for 10 min, these solutions were poured into a Teflon-lined stainless-steel autoclave, to which 2 g of P25 titanium dioxide was added. The synthesis mixtures were heat treated at 180 °C for 24 h (SrTiO₃_I), 48 h (SrTiO₃_II) or 72 h (SrTiO₃_III). Once they cooled down to room temperature a 5 M hydrochloric acid (HCl) solution was used to set the pH to 7. Based on the publication of Jiang et al., $0.625 \text{ M Sr}(\text{NO}_3)_2$ solutions were prepared, which were added to 6 M (SrTiO₃_IV) 12 M (SrTiO₃_V) and 18 M (SrTiO₃_VI) potassium hydroxide solutions each containing 5 mmol of P25 titanium dioxide [5]. The synthesis mixtures were transferred into Teflon-lined stainless-steel autoclaves and heat treated at 180 °C for 12 h. Subsequently, the materials obtained were washed with Milli-Q water and ethanol four times. To reduce carbonate content, SrTiO₃_VI was additionally subjected to HCl treatment (0.01 M) for 2 h (SrTiO₃_VII).

X-ray diffraction (XRD) measurements were carried out with a Rigaku Miniflex II diffractometer to determine the crystalline composition of the samples using the following parameters: $\lambda_{Cu K\alpha} = 0.15406$ nm, 30 mA and 40 kV. Scanning electron microscopy (SEM) measurements were performed with a Hitachi S-4700 Type II microscope applying 10 kV acceleration voltage. Transmission electron microscopy (TEM) measurements were also carried out, with a FEI Tecnai G2 20 X-Twin microscope, to investigate the morphology of SrTiO₃ photocatalysts.

The photocatalytic activity of the samples was evaluated by the photocatalytic reduction of CO₂. Analyses of the gases were performed with an Agilent 4890D gas chromatograph (GC). For the measurements a 2-m-long 0.25-inch-wide column containing Porapak QS was used, which enabled the complete separation and determination of the reactants and products.

Results and discussion

The XRD patterns of the photocatalysts are shown in **Fig. 1**. The patterns could be attributed to the cubic phase of the SrTiO₃ perovskite structure (JCPDS 00-035_0734). It was ascertained that the synthesis conditions (i.e., synthesis time and pH) did not have a significant influence on the crystalline structure of the samples. However, the application of HCl treatment for SrTiO₃_VII resulted in the remarkable reduction of carbonate (SrCO₃) content. As the amount of carbonate is an uncontrollable result of the synthesis it was beneficial to remove it in order to get a more clear view about the real activity of these materials.



Figure 1. XRD patterns of SrTiO₃ photocatalysts.

Since all photocatalysts showed similar morphology, a SEM and TEM micrograph representative of them are shown in **Fig. 2**. It was ascertained that samples of perfect 3D cubic

morphology were obtained with an average size of 65 nm (calculated based on the TEM micrographs).



Figure 2. A typical SEM (left) and TEM (right) micrograph of the SrTiO₃ photocatalysts.

The results of photocatalytic evaluation obtained by GC measurements are shown in **Fig. 3**. It was observed that the samples with the lowest carbonate content (that is, $SrTiO_3_VI$ and $SrTiO_3_VII$) had the best photoreduction activity. Comparing the samples synthesized with ($SrTiO_3_VII$) and without ($SrTiO_3_VI$) additional HCl treatment, it was ascertained that including it was beneficial for the photocatalytic activity. This result is in good agreement with the results obtained by XRD measurements.



Figure 3. Photocatalytic reduction of CO₂ via as-prepared SrTiO₃ photocatalysts.

Conclusions

The applicability of $SrTiO_3$ was successful in the reduction of CO_2 , resulting dominantly CO. The removal of $SrCO_3$ proved to be beneficial as the best sample was carbonate free, which was achieved with the addition of HCl. The morphology of the particles was cubic, with 50 nm as the particle size. No doping or other kind of structural modifiers were detected in the investigated samples.

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CHARACTERIZATION OF PRODUCTS FORMED DURING HYDROTHERMAL TREATMENT OF WINERY-WASTE BIOMATERIAL

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Abstract

Wine production remains one of the most important agricultural activities in the world, however, leads to the production of large quantities of winery-waste biomaterial. It has been estimated that around 25 kg of residues derive (around 70% grape skins, 12% stalks and 18% seeds) from 100 kg of grapes [1]. It is known that the waste biomaterial from the winery contains large amounts of various functional components with different bioactive properties (antioxidants, polyphenols, biosurfactants, anthocyanins, dietary fiber, etc.). Therefore, the wine industry encourages alternatives for recycling their waste biomaterials in order to profit from waste through their use as raw value-added materials. One of the technologies that achieve the reuse of bio-waste is the hydrothermal process especially suitable for the treatment of wet waste. Hydrothermal treatment of bio-waste takes place at high temperature and pressure operation conditions (200-400°C and 4-25 MPa) under sub/super-critical water system. A solid phase enriched in carbon (hydrochar, HC), a liquid phase with dissolved organic compounds and a small quantity of gases are separated as primary products of reaction [2,3]. In this paper, the characterization of HC and liquid phases formed during hydrothermal treatment of winerywaste biomass is performed. The HC was synthesized in a commercial stainless steel reactor by hydrothermal carbonization of black grape bio-waste at 200°C under auto-generated pressure of about 1.2 MPa for 2 h. The yield of HC was 44%. The general characteristics of the synthesized HC were determined. In the liquid phase, the total polyphenol content and antioxidant activity were determined. The results of the gravimetric analysis showed that HC has almost equal presence of moisture and dry matter (41% and 59%, respectively). The largest part of dry matter is volatile organic matter (56%), while the ash content is 3%. The liquid phase (2 mg/ml of dry extract in methanol) has high polyphenols content (0.66 g gallic acid/L) and showed a high antiradical potential (75% against DPPH free radical).

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ANTIOXIDANT ACTIVITY OF LIQUID PHASE GENERATED DURING WOOD-BIOMASS HYDROTHERMAL TREATMENT

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Abstract

There is a strong need to offer renewable alternatives for products heavily relying on fossil resources, contributing to a more sustainable economy and society. Biomass is a valuable feedstock of wide range renewable carbon-based products; its conversion paths must be carefully chosen in order to enable technologically feasible and cost-effective solution. One of the very rapid and efficient method to convert biomass into different multifunctional products is hydrothermal carbonization (HTC). HTC is a process of biomass conversion into solid product (hydrochar), bio-liquid (potentially a source of various valuable chemicals) and gaseous (combustible) product, utilizing the water in subcritical state (at 200-300°C). The liquid phase that separates after the production of hydrochar has a high content of organic matter that originates from the treated plant material. The liquid phase is usually rich in polyphenols, flavonoids, anthocyanins, sugars, amino acids, as well as the breakdown products of lignocellulosic materials. It is known that these compounds exhibit different bioactive properties: therefore the liquid phase after hydrothermal treatment has potential antioxidant properties. The composition and properties of the liquid phase largely depend on the starting biomass and on the reaction conditions of the process, and can be widely used in the agronomic, chemical, and pharmaceutical industries. The aim of this research is to examine the content of total polyphenols in the liquid phase after the hydrothermal carbonization under different reaction conditions and to determine its antioxidant activity. Additionally, the qualitative characterization of volatile compounds in the liquid phase was performed by gas chromatography coupled to mass spectrometry (GC/MS). The liquid phase was collected after HTC treatment of waste wood-derived biomass under different reaction conditions: temperature (200-300°C), autogeneous pressure (1.5-8.7 MPa) and reaction time (30-165 min). The liquid phase samples have high polyphenol content, which was influenced by the reaction conditions of the hydrothermal process, and was in the range 0.37-0.98 g GA/L. The highest polyphenol content was recorded in samples obtained at the temperature of 300°C. Also, the samples showed a high antiradical potential (according to DPPH assay), which is connected with the high polyphenolic content. Liquid fractions, in the concentration of 2 mg/mL, exhibited antioxidant activity in the range of 61.4-87.0% against DPPH free radical. In the liquid samples phenol and their derivatives dominated, which were formed as a consequence of lignin degradation. The performed analysis of the liquid phase obtained in the hydrothermal carbonization of wood-based biomass showed that such product is rich in bioactive components, particularly those with antioxidant capacities, proving its high potential for applicability against oxidative stress.

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REPELLENT EFFICACY OF AZADIRACHTIN ON *IXODES RICINUS* TICKS (ACARI, IXODIDAE)

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Abstract

The repellent efficacy testing, as well as the search for the most efficient and economically most justified preventive measures, emerge from the fact that in the last few years a zoogeographic expansion of certain tick species has been observed, in addition to an increased frequency of previously registered and emergent tick-borne diseases. The aim of this study was to compare the repellent efficacy of different azadirachtin essential oil concentrations as a function of time on *Ixodes ricinus* ticks. Azadirachtin essential oil had more than 50% repellent efficacy on *I. ricinus* three minutes after the application, and it decreased with time when all tested concentrations were concerned. Eight hours after repellent administration, concentrations of 50% and 75% have had repellent effects of 50%, while pure essential oil (100%) showed a repellency of 63.33%. In order for people to spend more relaxed and free time in nature, it is necessary to apply protective measures against ticks and one of the recommended ways is to use the substances that could be applied to the skin or clothes, and which have a repellent effect, such as azadirachtin essential oil.

Introduction

Urban ecosystems and rural areas are usually covered with fragmented green areas (parks, lawns, picnic areas, sports and recreation areas, meadows, forests, river banks), consisted of different vegetation types and structures. The specific floristic composition, the presence of diverse animal species suitable as the potential ticks' hosts, and the appropriate microclimatic conditions make these areas optimal habitats for the appearance, maintenance and increase of different tick species populations. Therefore, in order to predict the potential risk of tick-borne diseases (TBDs), both for humans and animals, it is necessary to distinguish the effects of a large number of abiotic and biotic factors in a certain habitat.

The lifespan of certain tick species can be more than three years [1]. During the warm months, from mid-June to the end of August, due to high temperatures and low relative humidity, ticks have reduced activity, and thus lower population density at some localities, which still does not decrease the risk of potential contact with these haematophagous ectoparasites. In average, the tick temperature optimum is 20-25°C, when more than 40% adults and 30% nymphs are active. The optimal value for relative air humidity is from 45 to 80%. The photoperiod is also an important environmental factor for tick activity [2]. Considering that, it can be concluded that the seasonal peaks of ticks increased activity, their occurrence in certain habitats, and their quest for an adequate host coincide with increased human activity in nature (sports, recreation, leisure, agricultural activities). The longer people spend their time in nature, the higher is the probability that direct contact with ticks will occur, and therefore, the possibility of various

pathogens transmission from ticks to humans. In the last ten years, more than 15 zoonotic pathogens and vector-borne diseases have been registered in Europe, of which, at least six are solely tick-borne: *Rickettsia* sp., *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Francisella tularensis*, Crimean-Congo hemorrhagic virus, and tick-borne encephalitis virus [3]. TBDs are very common in Europe and the United States. According to Mysterud et al. [4] about 300,000 people in the United States and 65,000 people in Europe are infected annually with tick-borne Lyme borreliosis. The registered TBDs in Serbia are: Lyme disease, human granulocytic anaplasmosis, tularemia, Q-fever, babesiosis, and rickettsial infections. However, it could be assumed that the real epidemiological situation is underestimated, because autochthonous cases of these diseases are registered sporadically, and the unified registration system at the national level has not still been established.

Testing the repellent and acaricidal efficacy of already registered and newly developed preparations, as well as finding the most efficient and economically most effective measures, emerge from the fact that in the last few years a zoogeographic expansion of certain tick species has been observed, as well as an increasing frequency of previously registered and emergent TBDs, all with the aim to protect humans and animals and prevent the possible pathogens transmission. Each step in the tick-host interaction can be interrupted, but only certain activities and processes can be counted as *sensu stricto* repellency which implies complete tick repellent from the host and/or its abandonment [5]. Therefore, the studies on the efficacy and repellency duration of certain compounds is of high importance and requires constant studying and methodology improvement, in order to monitor trends in the new synthetic and natural repellents development, the adequate tests application to assess repellency and finally, introduction and application of economically viable and widely available commercial preparations that are not harmful to human and animal health. Azadirachtin, the main bioactive ingredient of neem seeds (Azadirachta indica A. Juss., 1830 neem tree), is a very potent insecticide that affects the nutrition, development and molting of various insect larvae at very low concentrations, however low concentrations of this oil either have no effect or are negligible when it comes to ticks [6]. Therefore, the aim of this study was to compare the repellent efficacy of different azadirachtin essential oil concentrations as a function of time on Ixodes ricinus ticks.

Experimental

Ticks were collected using the flag-hour method [7]. Collected specimens were placed in plastic containers with perforated lids to provide sufficient ventilation, with a piece of cotton wool moistened with water to prevent the ticks' desiccation. Collected ticks were determined up to species level according to the identification keys [8].

The repellent efficacy of azadirachtin essential oil was determined according to the methods proposed by Kröber et al. [9] and Adenubi et al. [10]. Azadirachtin essential oil was administered in the form of Azadiroko Neem Cake[®] (derivative of *Azadirachta indica* seed), manufactured by BioGenesis. For the purposes of the experiment, the preparation was diluted with distilled water in five concentrations: 10, 25, 50, 75 and 100% and applied to ticks in three replicates. The control group was treated with distilled water. The bottom of Petri dishes (90 x 14.5 mm) was covered with filter paper on which three fields were drawn: zone without repellent, neutral - initial zone and the zone with repellent. Each tick was exposed to the repellent for 3 minutes. The repellent efficacy was determined by ticks movement away or in the direction of the administrated preparations, and calculated according to Thorsell et al. [11] and Tunón et al. [12], immediately after placing the ticks in the Petri dishes, and then after 1, 2, 4 and 8 hours. After each test cycle of 3 minutes, ticks were removed, and Petri dishes were stored in the laboratory, at a constant temperature of 20-22°C and a relative humidity of 40-

43%. The obtained results were statistically analyzed by ANOVA and Fisher's LSD test using Statistica 14.0.0 (TIBCO, University license).

Results and discussion

The average repellent efficacy was calculated for five different concentrations, three replicates, and five time intervals and presented in Table 1.

Concentrations	Time intervals							
Concentrations	3 minutes	1 hour	2 hours	4 hours	8 hours			
10%	53.33	50.00	43.33	30.00	13.33			
25%	63.33	60.00	50.00	50.00	40.00			
50%	70.00	60.00	53.33	53.33	50.00			
75%	83.33	73.33	63.33	60.00	50.00			
100%	93.33	86.67	80.00	70.00	63.33			

Table 1. The average repellent efficacy

The obtained results are in accordance with the previously published data, where neem seed essential oil in a concentration of 40% was the most effective and with the highest toxicity, while a concentration of 10% showed the lowest toxicity [13]. The same study emphasized that higher concentrations showed a higher mortality rate of *Rhipicephalus pulchellus* ticks compared to the lower concentrations, which is similar to our results, where higher concentrations had higher repellent effects on *I. ricinus* ticks. The same authors recommended the concentration of 30% of neem seed essential oil as the most efficient and economically justified [13], although our results highlighted concentrations over 50%.

Based on the obtained results, it can be concluded that azadirachtin essential oil had more than 50% repellent efficacy on *I. ricinus* after three minutes of application, and it has decreased with time when all tested concentrations were concerned. Eight hours after repellent administration, concentrations of 50% and 75% have had repellent effects of 50%, while pure essential oil (100%) showed a repellency of 63.33% (Table 1). Application of pure azadirachtin essential oil to the rabbits provided them complete protection against *Amblyomma variegatum* bites for 2 to 4 days [14]. Statistical analyses of the obtained results indicated that different azadirachtin essential oil concentrations had high statistical differences in relation to the repellent efficacy as a dependent variable (p=0.000559 for p<0.01) (Graph 1.). Contrary, the time intervals were not statistically significant (Graph 2). The application of Fisher's LSD test defined the statistical significances among the applied concentrations (Table 2).

Concentrations	Mean	Statistical significance					
10%	37.998	а					
25%	52.666	a,b					
50%	57.332	b					
75%	65.998	b,c					
100%	78.666	С					

Table 2. The results of the Fisher's LSD post-hoc test

Azadirachtin essential oil had a significant repellent effect on *I. ricinus* if concentrations of 50% and more were used. However, azadirachtin, in addition to its repellent action, has a pronounced acaricidal, ovicidal and larvicidal efficacy on different tick species, as it interferes with the tick metamorphosis [15]. Azadirachtin affects arthropod chemoreceptors, preventing nutrition and oviposition, as well as meiosis, muscle physiology, digestive functions, and biological rhythms

[13]. Its primary mode of action is through prothoracicotropic hormone (PTTH) and allatotropin inhibition. Obstruction of these morphogenetic peptide hormones disrupts the secretion of ecdysone and juvenile hormones in hemolymph, which affects metamorphosis, reproduction, and development.



Graph 1. Repellent efficacy depending on different concentrations

Graph 2. Repellent efficacy depending on time intervals

Higher concentrations of azadirachtin had an ovicidal activity on fertilized eggs of Hyalomma anatolicum excavatum, as well as an acaricidal effect on adults who did not adopt a blood meal in the first 24 hours after application [15]. Azadirachtin had a larvicidal effect that depended on the concentration and time of application, with a 100% mortality observed at concentrations of 20, 40, 60, 80 and 100% after 24 hours on Boophilus decoloratus larvae [15]. Moreover, azadirachtin had an acaricidal effect on larvae, nymphs, and females of A. variegatum, where mortality increased with higher concentrations [14]. In addition, the neem seeds essential oil significantly reduced the number of hatched eggs of *R. appendiculatus* and *B. decoloratus*, where the reduction was higher in eggs treated with undiluted (100%) compared to diluted oil (25%) [14]. Azadirachtin essential oil can be used with certainty to control ticks in animals, as there are no registered side effects [16]. Moreover, neem essential oil has fungicidal and bactericidal properties, which can act on secondary infections caused by tick bites. Neem essential oil with its component azadirachtin can also be used to control ticks in livestock, as it is a cheap and natural acaricide that could also play a significant role in reducing the nonselective use of synthetic chemicals that are potentially dangerous for humans, animals and environmental health [17]. The azadirachtin essential oil can be sprayed on pastures and meadows intended for cattle grazing, which would reduce the ectoparasites infestation of domestic animals and the potential pathogens transmissions that could endanger human and domestic animals health [14].

Conclusion

Azadirachtin essential oil had more than 50% repellent efficacy on *I. ricinus* three minutes after the substance application, but it decreased with time when all tested concentrations were concerned. Eight hours after repellent administration, concentrations of 50% and 75% have had repellent effects of 50%, while pure essential oil (100%) showed a repellency of 63.33%. In order for people to spend more relaxed and free time in nature for rest and various sports and recreational activities, it is necessary to apply preventive protection measures against obligatory haematophagous ectoparasites such as ticks. One of the prevention methods is the use of the substances with confirmed repellent efficacy, which could be applied to the skin or clothes, such as azadirachtin essential oil.

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CHEMICAL CHARACTERIZATION OF CHERRY FRUITS

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Abstract

The variability of biochemical components in fruits is relatively high, due to the many varieties of each species, applied technology and not least due to environmental conditions.

The sweet cherry (*Prunus avium L.*) and the sour cherry (*Prunus cersus*) are among the most popular fruits. The origin of *Prunus* genera is in the Asian continent, and they produce fruits and hardwood. Cherries are characterized by their attractive appearance and delicious tastes. Cherry fruit is especially consumed fresh but can be processed as jam, wine, juice, dried fruit, candy, and other processed products. The rich nutritional content and a broad range of bioactive compounds are important qualities of cherries.

The aim of this study was to establish the chemical composition of cherry fruits (*Prunus avium* and *Prunus cerasus*) grown in the West Region of Romania. In this study was investigated total soluble solids content, total polyphenols content, total antioxidant capacity.

Content of total phenols and total antioxidant capacity from cherries were estimated by spectrophotometric methods.

Keywords: cherry species, total polyphenols, antioxidant capacities, CUPRAC method

OPTICAL AND ANTIOXIDATIVE PROPERTIES OF 5-(2,6-DIMETHYLPHENYL)-6-HYDROXY-4-METHYL-2-OXO-1,2-DIHYDROPYRIDINE-3-CARBOXAMIDE

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Abstract

A new heterocyclic azo dye 5-(2,6-dimethylphenyl)-6-hydroxy-4-methyl-2-oxo-1,2dihydropyridine-3-carboxamide was synthesized and structurally characterized by elemental analysis, FTIR, ¹H and ¹³C NMR spectroscopy techniques.

These analysis have confirmed that synthesized dye exists in the *tinctorial strongest* tautomeric *form*, hydrazone form. The dye is characterized by reflection spectrum, while absorption and emisson spectra are recorded in nine different solvents.

The antioxidant activity of the synthesized dye has been chemically tested and has been shown to have great potential as an antioxidant molecule.

Introduction

Azo compounds derived from 2-pyridone have been a special center of attraction due to their wide fields of application such as textile, pharmaceutical, electronic and graphic industries.

Azo dyes are often used in the field of non-linear optics, dye fibers, optical storage data and dye sensitized solar cells [1,2].

A thorough knowledge of molecular structure is important for finding the structure-activity relationship.

This study describes the synthesis, molecular structure, solvatochromism, and evaluation of antioxidant activity of $5-(2,6-\text{dimethylphenyl})-6-\text{hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide (Fig. 1). The dye molecular structure was confirmed based on the results of elemental analysis, FTIR, ¹H and ¹³C NMR spectroscopy. The optical properties of the newly synthesized dye was defined on the basis of UV/Vis spectroscopy by recording the reflection spectra and determining the color position in the chromaticity diagram. Considering that dyes generally need to be dissolved in order to combine with other materials, knowledge of their behavior in solutions is of great importance, and therefore the absorption and fluorescence spectra of dye solutions in solvents of different polarity were recorded. Antioxidative activity of the dye has been evaluated by ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay, expressed as IC₅₀ value and compared to ascorbic acid.$

Experimental

Synthesis

The investigated azo dye have been synthesized from the 2,6-dimethylphenyl diazonium salt and 6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide [3], using the classical reaction of diazotization and diazo-coupling [4]. The crude product was recrystallized from N,N-dimethylformamide. Elemental analysis was performed using a Vario EL III elemental analyzer. The IR spectra were recorded using a Bomem (Canada) MB-Series 100 Fourier

transform-infrared (FT-IR) spectrophotometer in the form of KBr pellets. The ¹H and ¹³C NMR data were performed using a Varian Gemini 2000 (200 Hz and 50 Hz, respectively) in deuterated dimethyl sulfoxide (DMSO- d_6) with tetramethylsilane (TMS) as an internal standard. All spectral measurements were carried out at room temperature (25 °C). The dye reflection spectra were recorded on a Shimadzu 2600 spectrophotometer in the range of 220-1350 nm.

The ultraviolet-visible (UV/Vis) absorption spectra were recorded on a Shimadzu UV-Visible UV-2600 (Japan) spectrophotometer in the range 200-700 nm, while emission spectra were recorded on Fluorescence spectrophotometer Perkin Elmer precisely (LS 45 Luminescence Spectrometer).



Fig. 1. *The chemical structure of the* 5-(2,6-*dimethylphenylazo*)-6-*hydroxy*-4-*methyl*-2-*oxo*-1,2-*dihydropyridine*-3-*carboxamide*

5-(2,6-dimethylphenylazo)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide: Orange crystalline substance: m.p.: 243-245 °C, yield 46 %; IR (KBr, v/cm⁻¹): 3162 (NH), 3384 (NH), 1672, 1643 (C=O); ¹H NMR (200 MHz, DMSO-*d*₆, δ/ppm): 2.24 (3H, s, CH₃), 2.48 (6H, s, CH₃), 7.16 (1H, t, J = 8,0 Hz, Ar–H), 7.24 (2H, d, J = 8,0 Hz, Ar–H), 7.55 (1H, s, NH₂), 7.75 (1H, s, NH₂), 11.75 (1H, s, NH on heterocyclic), 14.62 (1H, s, NH of hydrazone form); ¹³C NMR (50 MHz, DMSO-*d*₆, δ/ppm): 166.9 (CONH₂), 162.3 (CO Py), 146.1 (Ar), 138.4 (Py), 130.1 (Ar+Py), 129.2 (Ar), 126.3 (Ar), 125.5 (Py), 124.3 (Ar), 19.7 (CH₃), 15.1 (CH₃). Anal. Calcd for C₁₅H₁₆N₄O₃: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.12; H, 5.43; N, 18.73.

Evaluation of antioxidant activity

Antioxidant activity of the dye is determined by ABTS assay [5]. The test is based on the ability of the molecule to scavenge the ABTS⁻⁺ radical cation. The precentage inhibiton was calculated acording to the equation:

Inhibition (%) = (Ac - As)/Ac * 100

where As is the absorbance of the sample solution and Ac is the absorbance of the control solution. Ascorbic acid was used as a standard antioxidant molecule. Furthermore, IC₅₀ values of the dye and ascorbic acid were determined. The methanolic solutions of dyes and ascorbic acid were prepared at concentrations 3, 1.5, 0.75 and 0.15 mM, and obtained IC50 were compared.

Results and discussion

Pyridone azo dyes bearing –OH group in the position 6 of the pyridone ring are known to exhibit azo-hydrazone tautomerism, in both solid state and solutions. The IR and NMR spectra of the dye clearly show the existence of the hydrazone form in solid state and DMSO- d_6 , respectively. The infrared spectra showed characteristic vibration of two carbonyl groups at

1672 and 1643 cm⁻¹ indicating the presence of the hydrazone tautomeric form. Also, N–H stretching vibration from hydrazone group is observed at the 3384 cm⁻¹, while N–H stretching vibration of pyridone moiety is noted at the 3162 cm⁻¹.

The ¹H NMR spectrum of dye exhibits a signal at 14.62 ppm. This signal corresponds to N–H proton resonance of the hydrazone form. Also, ¹³C NMR spectra confirmed the existence of the hydrazone form. Peak observed in the 166.9 ppm is ascribed to carbonyl group of 3-amido group, while peak originating from the C atom of carbonyl group, in the pyridone ring is observed at 162.3 ppm.

Reflection spectra

Figure 2 shows the reflection spectra, the photographed dye and its position in the color system CIE lab. The CIE lab color system determines color based on the dominant wavelength and mean reflectance [6].



Fig. 2. Reflection spectra of dye and its position in CIE lab color system

The absorption and fluorescence spectra of the dye were recorded in solvents of different properties in order to see the influence of the solvent on the position of the absorption and fluorescent maxima, as well as on the shape of the bands. The strongest absorption band can be seen in the region from 350 to 500 nm, and corresponds to the π - π * transition of hydrazone form. The absorption maxima of the dye show little variation in different solvents. The Stokes shift in all tested solvents is about 80 nm.



Fig. 3. Apsorption and emission spectra of dye in different polarity solvent

Antioxidative properties

Antioxidant activity of the dye is assayed using the ABTS method. The scavenging activity of azo dye was compared to the activity of ascorbic acid. The results have shown that dye exhibit remarkable activity (c = 3 mM) of the investigated dye (96.0%) compared to ascorbic acid (95.3%). Furthermore, IC₅₀ values, which corresponds to the concentration of sample able to scavenge 50% of ABTS radicals in the solution, are evaluated. IC₅₀ values of the dye (0.78 mM) and ascorbic acid (1.25 mM) have shown that the dye is more potent antioxidant molecule than ascorbic acid (Fig. 4) and could be considered as promising antioxidant candidate.



Fig. 4. IC₅₀ values of the dye and ascorbic acid

Conclusion

The investigated dye was synthesized and according to the FTIR, ¹H and ¹³C NMR spectral data, dye exists in the hydrazone form in the solid state and in DMSO- d_6 . Analysis of the UV/Vis spectra confirmed that the analyzed dye appears in all solvents in hydrazone tautomeric

form, while the analysis of emission and absorption spectra determined that the Stokes shift in all tested solvents is about 80 nm.

Evaluation of the antioxidant activity of the dye and ascorbic acid have shown that dye is more potent scavenger of ABTS⁺⁺ radical cation than the stanard molecule. Furthermore, its IC50 value is lower than IC50 value of ascorbic acid indicating that this dye could be considered as promising antioxidant candidate.

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ANTIOXIDANT AND PROOXIDANT FEATURES OF N-CQD IN PHOTOCATALYTIC TESTING OF AQUATIC MEDIA

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Abstract

The modern age science is still searching for an effective photocatalytic material for the treatment of colored discharges from different industries which cause severe environmental issues. The excellent properties of nitrogen doped carbon quantum dots (N-CQD) enable their successful application as photocatalytic material in organic dye removal triggered under light absorption. With this in mind we first present a successfully performed microwave-assisted synthesis method, a green, simple and economically affordable method for N-CQD synthesis with high nitrogen percentage incorporated in the form of pyrrolic, pyridinic/NH₂ and graphitic/NH₃⁺ groups. The pro-oxidant and antioxidant features of the synthesized N-CQD were further presented, with high removal efficiency of synthesized N-CQD towards the methylene blue (MB) organic dye, as one of the leading water pollutants with a major risk to aquatic and human life.

Introduction

The MB dye, is extremely dangerous with strong effect on human beings as well as on the environment [1–3]. Efforts for removal of organic toxic dyes from natural sources, through adsorption or in the presence of photoactive materials, over the years caught significant research attention [4,5]. Due to ease functionalization, optical properties and high stability upon irradiation, CQD found application in treatment of different pollutants [6]. N-doped CQD are interesting carbon nanoparticles with particle sizes less than 10 nm and outstanding photoluminescent properties. The presence of oxygen containing functional groups provides a good solubility of N-CQD in water media allowing their application as photocatalyst in organic dyes treatment.

Guided by the idea that 25% of sunlight is actually a blue light, we explored the time dependent photoactivity of N-CQD towards removal of MB dye from water under blue light irradiation (470 nm). Apart from efficient production of hydroxyl radicals (•OH), we discovered the potential antioxidant activity of synthesized N-CQD.

Experimental

The N-doped CQD were synthesized using green precursor and microwave assisted method as described previously [7]. The antioxidant potential of N-CQD was measured using DPPH•. The freshly prepared methanol solutions of DPPH• was mixed with different concentrations of N-CQD (20–200 μ M) water solution in total volume of 1200 μ l. Samples were incubated for 30 min in dark at room temperature, followed by monitoring of the DPPH• absorption at 515 nm by UV-Vis absorption spectrometry. Evaluation of the antioxidant activity of N-CQD examination by KMnO4 reduction assay was performed following the protocol from Ruiz et. al [8]. After incubation in dark conditions for 1h, the change in the absorption intensity of charge transfer transitions at 506, 525, 545 and 566 nm, along with the solution color change was monitored. The prospect of hydroxyl radicals (•OH) to be the ROS in charge for removal of

MB organic dye was examined using the test with terephthalic acid (TPA) by photoluminescence (PL) [7].

The MB removal efficiency in the presence of the N-CQD photocatalyst was examined in batch under blue light irradiation (470 nm). The reaction mixture containing 20 ml of MB stock solution (0.03 mM) in the presence of a photocatalyst the N-CQD (0.25 mg/ml) was exposed to irradiation in black box conditions to avoid the influence of the external light. Samples were placed directly on the free-standing lamp with free surface area of 28 cm² and irradiation power of 3 W, followed by irradiation in different time intervals 120, 180, 240 and 300 min. The removal efficiency was monitored by decrease in the absorption band maximum at 664 nm characteristic for MB dye.

Results and discussion

The N-doped CQD received attention from the research community with regard to their proand antioxidant activity. As 0D nanomaterials, the CQD are known as both electron donors and acceptors. Hence, along with the ability to generate ROS under UV-Vis light irradiation they express an antioxidant activity as well [9].

The capacity of N-CQD to produce hydroxyl radicals was tested using luminescent molecule such as TPA. The TPA reacts with •OH forming highly fluorescent product HTPA under irradiation. The PL peak intensity of the HTPA is proportional to the amount of produced •OH radicals. In Figure 1 we present the PL intensity change of suspension containing N-CQD photocatalyst and TPA transformation into hydroxylated HTPA form. No peak was noted in the absence of the photocatalyst, while the presence of N-CQD photocatalyst promoted the reaction of TPA with •OH radicals on the photocatalyst-water interface.



Figure 1. The pro-oxidant activity of N-CQD using TPA test (A) and the TPA transformation into fluorescent form HTPA the presence of N-CQD (B).

The antioxidant potential of CQD has been proven previously using DPPH as most common free radical for evaluation of N-CQD scavenging activity (Figure 2) [8]. The Vit. C with high scavenging activity for reactive oxygen species in the same concentration range was used as a control (Figure 2B). Results for control Vit. C showed the increasing activity at concentrations up to 80 μ g/ml followed by saturation effect, while the sample N-CQD showed only moderate efficiency bellow 80 μ g/ml. The actual scavenging activity increase was observed for concentration 100 to 200 μ g/ml. Further increase in the concentration would lead to saturation effect (Figure 4D). Observation was followed by the color change from purple to yellow. The estimated amount of antioxidant necessary to decrease the concentration of DPPH by 50 % was calculated to be 33.11 μ g/ml for Vit. C, while for the N-CQD the value of 177.82 μ g/ml showed five times lower efficiency comparing to control (Figure 2C).



Figure 2. The scavenging activity of N-CQD (A) and control Vit. C (B) against DPPH free radical; the calculated IC_{50} for both set of samples (C), and DPPH• transformation into its stable form DPPH₂ in the presence of N-CQD (D).

The evaluation of the oxide radical scavenging activity of N-CQD with the KMnO₄ reduction assay is presented in Figure 3A. Following the obvious decrease in the characteristic KMnO₄ band intensity at 506, 525, 545 and 566 nm by adding N-CQD to the KMnO₄, the spectral changes confirmed reduction of Mn⁷⁺ to Mn²⁺. At a concentration of 0.04 mg/ml for N-CQD, a strong decrease in absorption of over 93 % was observed followed by saturation effect above 0.06 mg/ml. Comparable results were obtained for control experiment in the absence of N-CQD using ascorbic acid (Vitamin C – Vit. C) as standard (Figure 3B). The obtained IC₅₀ value for sample set with N-CQD was 0.02 mg/ml, while for control experiments in the presence of Vit. C the IC₅₀ value was comparable (0.01 mg/ml), suggesting the extraordinary antioxidant activity of N-CQD (Figure 3C). The high sp² to sp³ ratio 18.9 at% to 22.7 at% in synthesized N-CQD stimulate the electron transfer from N-CQD to KMnO₄ promoting the reduction.



Figure 3. The KMnO₄ reduction assay of: N-CQD (A) and control Vit. C (B); calculated IC_{50} values for both set of samples (C), and KMnO₄ reduction to colorless Mn^{2+} in the presence of N-CQD (D).
Organic dye MB, as a member of phenothiazine family, possess a heterocyclic aromatic chemical structure with three combined aromatic rings and C–S, CN and CN= functional groups. As a water soluble molecule, it can slowly degrade in aqueous media due to •OH produced from water becoming active upon irradiation [6]. The MB removal process started in the presence of synthesized N-CQD in dark conditions (14 %) as a result of the MB dye adsorption on N-CQD high surfaces contact area (Figure 4A). After switching on the irradiation source, the removal efficiency of the synthesized N-CQD photocatalyst increased exponentially with time as shown in Figure 4B. Under blue light irradiation, the MB removal process became faster in the presence of N-CQD as photocatalyst indicating their strong influence on the photocatalytic activity. This is due to the fact that N-CQD possess unique lone pair states that are contributed by nitrogen atoms, which affects their band gaps leading to formation of new energy levels in between the conduction and the valence bands [10].



Figure 4. The photocatalytic test results (A), and the N-CQD removal efficiency percentage towards MB dye against irradiation time (B).

Upon irradiation of N-CQD, the hydroxyl radical species ('OH) can be generated. Therefore, the degradation of MB dye goes preferably through decomposition of the chromophore structure and the destruction of the homo and heteropoly aromatic rings of MB molecule [11]. The initial step of MB degradation is cleavage of the bonds of the C–S⁺=C functional group in MB dye molecule [12]. The adsorption of MB dye onto photocatalyst leads to interaction of the generated OH• radicals with C–S⁺=C functional group in cationic dye. In order to maintain double bond conjugation, the central aromatic ring, containing S and N atoms, will open. Further attack of reactive radical species leads to dissociation of the two benzene rings into intermediate products, whose successive hydroxylation further leads to the aromatic ring opening and finally to the conversion of organic carbon into harmless gases [6].

Conclusion

The presented research showed the green, easy and fast microwave assisted method for production of N-doped CQD from a glucose precursor. Synthesized N-CQD, at low concentration of only 0.25 mg/ml, showed remarkable removal efficiency towards MB dye under blue light irradiation. In contribution, the high percentage of nitrogen, in the form of amino groups or incorporated in the basal plane of N-CQD, resulted in synthesis of electron-rich structures, with oxide radical scavenging activity to be expected. By increasing the photocatalyst concentration and fine-tuning of the reaction media, the MB dye adsorption onto the N-CQD free surface can be promoted. Consequently, the reduction of irradiation time needed for the same removal rate is expected, which opens the possibilities to further expand the research focus.

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INFLUENCE OF ZINC OXIDE ADDITION ON LITHIUM-NIOBIUM-TITANIUM OXIDE CERAMICS

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Abstract

Lithium-niobium-titanium oxide ceramics (LNTOs) are prepared by solid-state reaction method and sintering at lower than standard temperatures for potential microwave electronics applications [1]. ZnO is added as a functional additive in two different weight percentages (2 and 5 wt. %) and changes in microstructure and electrical properties are observed. Scanning Electron Microscopy (SEM) has confirmed the existence of plate and rod-like shaped particles, which is a characteristic property of the *M*-phases group of LNTOs [2]. The density increase and porosity reduction appeared also as an effect of ZnO addition. X-Ray Diffraction (XRD) patterns and Raman spectra have been used to identify phases present in the synthesized LNTOs and to investigate their structure. To understand the conduction mechanism, electrical properties associated with microstructures in synthesized LNTOs were studied as a function of frequency and temperature using the complex impedance spectroscopy (IS) technique. The analysis of impedance data using an equivalent circuit indicates the differences in the electrical properties of the two LNTOs that are mainly attributed to the processes related to the grain boundaries.

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GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF IRRADIATED FLUOXETINE AQUEOUS SAMPLES

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Abstract

The last decade witnessed the drastic increase in the use of antidepressant drugs, being fluoxetine the most prescribed worldwide. Conventional wastewater treatment is inefficient in removing fluoxetine and its accumulation in water bodies and water living organism is inevitable. Among several methods for contaminant removal from wastewater, electron beam irradiation is an efficient and green technology. This work presents the characterization of aqueous fluoxetine samples before and after irradiation. Gas chromatography coupled to mass spectrometry was used to identify the original compound and its irradiation products. Results indicate a drastic reduction in fluoxetine presence after the irradiation process. Radiolysis pathways were proposed based on mass fragments identification.

Introduction

The detection frequency of contaminants of emerging concern (CEC) has increased over the past decade [1]. Although their potential environmental hazard has been confirmed by many authors, toxicity data is still scarce. Among CECs, antidepressant drugs are usually a major contaminant found in different water matrices, from wastewater to agricultural irrigation systems [2] and groundwater [3]. According to the Organization for Economic Cooperation and Development (OECD), the consumption of antidepressants has increased 60% worldwide in the last decade [4].

Antidepressants are a category of psychiatric drug used in the treatment of depression, anxiety, panic disorder and obsessive-compulsive disorder. Among these, fluoxetine (**Figure 1**) is the most prescribed antidepressant in the world.

Approximately 10% of the parent compound is eliminated through urine, entering, thus, the water treatment facilities as human waste, mainly. Fluoxetine is a hydrolytic and photolytically stable drug with a long half-life, which accumulates in biological tissues [5].



Figure 1 –Fluoxetine molecular structure

Since fluoxetine removal in wastewater treatment plants is inefficient, some alternative treatments have been developed. Several degradation methodologies for fluoxetine have been reported, such as sorption, biodegradation, photodegradation, oxidation and irradiation. Electron beam irradiation (EBI) treatment is a green technology, being efficient and safe. The

radiolysis of water molecules produces reactive species, as shown in Equation 1, which promote the degradation of compounds [6].

$$H_2 O \xrightarrow{EB} e_{(aq.)}^- + H^{\bullet} + HO^{\bullet} + H_2 O_2 + H_2 + H_3 O^+$$
 (Eq.1)

Experimental

Irradiation of fluoxetine aqueous samples: Standard solution of pure fluoxetine was prepared at 100 ppm. Next step followed the batch scale irradiation that was carried out at a 1.4 MeV electron beam accelerator, by means of the variation of electric current. A Shimadzu Co. gas chromatograph/mass spectrometer model QP2020 NX was used, and the instrumental parameters were: Restek Rtx-5MS capillary column, injector temperature 250°C, column flow 0.9 mL/min, split ratio 5.0, oven temperature started at 140 °C and was raised to 300 °C (40 °C/min) and held for 2 minutes, ion source temperature 280 °C and interface temperature 280 °C, positive electron impact ionization mode.

Results and discussion

Chromatographic results (**Figure 2**) indicate the presence of only one irradiation product after 1 kGy.



Figure 2 – Chromatogram of irradiated fluoxetine

As indicated by Shao [7], this dose (1kGy) degrades 98% of the active fluoxetine. **Figure 3** presents a comparison of the same sample, before and after irradiation. The peak area of the irradiated sample is negligible (10^3 times less intensity).



Figure 3 – Chromatogram before and after irradiation

Shao *et al.* [7] proposed degradation pathways for fluoxetine under EBI, using liquid chromatography coupled to tandem mass spectrometry (LC/MS-MS) using electrospray ionization (ESI) mode. Similarly, Silva *et al.* [8] used direct injection on an ultra-high resolution Qq-time-of-flight (UHR-QqTOF) mass spectrometer on ESI mode as well. Despite employing the same characterization technique (MS), this work differs from both Shao and Silva for the ionization mode. Electron (impact) ionization is a 'hard' ionization mode, since the high energy provided by electron at 70 eV generates several fragments. Electrospray is considered a 'soft' ionization mode. Also, some fragments might be the product of fragment recombination, which makes their identification much more complicated.

The mass spectrum of fluoxetine presents 4 characteristic fragments (44, 104, 148, 162, 309) Mass spectrum of the irradiated product (**Figure 4**) indicates the presence of 8 fragments (44, 73, 143, 207, 253, 281, 327, 341). The only fragment present on both samples, before and after irradiation, is the base peak (m/z 44).



Figure 4 – Mass spectra of irradiated fluoxetine

Based on fragments presented on **Figure 4**, we propose that OH• is the most reactive species, attacking both the carbon oxygen bond, yielding fragment m/z 73, and the aromatic ring, yielding fragment m/z 327 and 341. The last reaction if the defluorination, yielding fragment m/z 143.

Conclusion

Mass spectrometry is a ubiquitous characterization technique in the field of environmental analysis. Nonetheless, the coupling with gas chromatography (GC) is ever less usual in comparison to liquid chromatography (LC), a more sensible yet highly expensive. This work established a methodology for the characterization of irradiated fluoxetine samples by GC/MS, a cheaper and more commonly employed analytical technique.

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COMPUTATIONAL MODELING OF DISTRIBUTION COEFFICIENT OF TRIAZINE DERIVATIVES AND ITS INFLUENCE ON THEIR CHROMATOGRAPHIC BEHAVIOR IN RP-UHPLC SYSTEM

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Abstract

In the present study, the distribution coefficient (log*D*) of a series of *s*-triazine derivatives with acyclic and cyclic substituents was modelled by using MarvinSketch software. The calculation of log*D* was done by Viswanadhan and Ghose (VG), Klopman's (KLOP) and PHYSPROP[©] (PHYS) methods. During the calculations, the tautomerization and resonance were taken into account. The pH-log*D* dependences were obtained for each molecule. The log*D* data determined at various pH values were further correlated with retention parameter (log*k*) of studied compounds. The log*k* values were determined by using revered-phase ultra-high performance liquid chromatography (RP-UHPLC) with C₁₈ stationary phase and mobile phase as a mixture of methanol and water. The volume fraction of methanol in the mobile phase varied from 50 to 85 (v/v). Based on the retention time and dead time, log*k* parameters were obtained. Considering the fact that log*D* parameter is in most cases a better descriptor of the lipophilicity of a compound than partition coefficient (log*P*), it can better describe the retention behavior in reversed-phase liquid chromatography that strongly depends on the compound's lipophilicity.

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DETERMINATION OF ORGANIC CARBON IN MINERAL AND THERMAL WATER

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Abstract

Total organic carbon (TOC) in mineral and thermal waters was measured and statistically evaluated. A calibration curve was constructed, and accuracy and reliability, as well as repeatability and reproducibility, were evaluated in pool waters. The results showed that the method is suitable for the determination of TOC in different thermal and mineral water samples. The results were statistically evaluated. The method was tested within interlaboratory comparison. The measurement deviations were up to 4 %, which ensures the accuracy and reliability of the results.

Introduction

The history of TOC determination dates goes back to the 1960s century. Development became faster when it was started to be used in the pharmaceutical industry.[1] TOC means the determination of all types of carbon in water, some of which can be oxidized by biological and chemical processes [2] The addition of chemicals to water samples might interfere with the measurement of TOC. In addition to TOC parameter, specific ultraviolet absorbance (SUVA) was also determined [3]. The high value of the SUVA indicator in the pond proved that high-molecular organic compounds are resistant to biodegradation.

The two most commonly used TOC analyzers for aqueous samples can be distinguished by their different oxidation methods, involving either high-temperature combustion or photochemical UV- persulfate oxidation [4] In high temperature combustion, the samples are oxidized at high temperatures above 933 K in the presence of catalysts, e.g. Pt.Carbon dioxide is determined by nondispersive infrared (NDIR) detector.

The main objective of the present study was to measure the total dissolved organic carbon in mineral and thermal waters. The same method is already used for drinking, surface, and underground waters' TOC determination. The analytical method includes optimization, calibration curve determination, assessment of the stability of the system, linear model verification, repeatability, and reproducibility of the determination.

Experimental

Shimadzu TOC-5000A and automatic sampler ASI 5000 are seen from Figure 1. The concentration range is from 4 ug/L to 4 g/L and volume of $10 - 2500 \mu$ L. Chloride ions interfere the measurement above 100 mg/L. Therefore, scrubber was used for removal of chloride ions, which could interfere with NDIR detector. Oxidation catalyst is used during catalytic oxidation, based on Al₂O₃ and Pt. There is no carbon remaining on the surface of catalyst.



Figure 1. Shimadzu TOC-5000A with automatic sampler (left)

Standard solution (Potassium hydrogen phthalate; M=204,23 g/mol; AR grade, Merck, Germany) was used for preparation of 1000 mg/L standard solution. Other standard solutions were prepared by proper dilution. Calibration was done with 0.3 0.6, 0.9, 1.2, 1.5 mg/L and 3.0 mg/L solutions.

Samples were taken into plastic bottles and kept at 275 K. The samples were filtered with 0.45 μ m membrane to eliminate the effects of the particulate organic carbon (POC) on the TOC value [5]

Results and discussion

Table 1 represents calibration curve data.

Tuble 1. Cultofullon cure unu (11 – urcu)						
Calibration points	TOC (mg/L C)	Α				
1	0.3	19097				
2	0.6	28049				
3	0.9	35601				
4	1.2	43420				
5	1.5	53705				

Table 1. Calibration cure data (A = area)

Calibration curve equation: Y=10616 + 28135xSlope: 10616 Intercept: 28135 The correlation coefficient $R^2 = 0.998$ The quality coefficient Qc = 2.2 %.

The results showed that calibration curve is linear.

Based on 9 repeated measurements at lower (TOC_1) and upper concentration ranges (TOC_u) the linearity test was performed. The measurements are shown in Table 2.

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Replicate number	TOC _l (mg/L C)	TOC _u (mg/L C)
1	18467	57822
2	19027	54708
3	16505	52533
4	16956	53225
5	18327	52720
6	19254	54123
7	19757	53267
8	19612	53633
9	19121	55106

Table 2	The measurement	$a \pm 0.2$	ma/L a	nd 15 ma/I	
Table 2.	The measurement	at 0.5	mg/L a	nu 1.5 mg/L	

Homoscedasticity was determined based on F test: calculated F-test value equaled 2.04, which was lower than tabulated F test value: F test (nl, nu, 99 %) = 6.03.

There were no outliers found following Grubbs and Beck test.

The determination of the LOD and LOQ is based on 9 replicates of blank measurements shown in Table 3.

Parameter	$A_b (mg/L C)$
1	8494
2	8054
3	7116
4	8876
5	7628
6	7909
7	7625
8	7546
9	7143

Table 3. Measurements of blank $(A_b = blank area)$

Limit of detection LOD was determined at 0.062 mg/L C.

Limit of quantification LOQ was determined at 0.5 mg/L, which is above first calibration point (0.3 mg/L).

Trueness was determined by measuring the recovery of TOC in waters.

n	Recovery (%)
1	100.70
2	95.26
3	88.12
4	84.18
5	101.60
6	93.28
7	120.10
8	88.96

 Table 4. Determination of trueness

The average recovery was calculated at 96.53 %.

T-test was performed to determine the accuracy. The tabulated value $t_{tab} = 2.365$ at 95 % confidential interval and calculated t = 0.871, which is smaller than tabulated t_{tab} , therefore the method is accurate.

Different samples were chosen for determining the reproducibility. TOC was measured in 9 replicates for each sample. The *RSD* was up to 4 %, which is much lower than desired 15 %. Thus reproducibility is very good.

TOC was measured in real samples of mineral and thermal water (in pools). Results are presented in Table 5.

Sample number	TOC (mg/L C)	_
1	2.06	
2	2.69	
3	6.54	
4	64.11	
5	2.42	
6	1.96	
7	1.65	
8	4.22	
9	80.78	

Table 5. The results of TOC measurement in water samples

Measured were values in outdoor (No4 and No9) and others from indoor pools. Higher values were measured in outdoor thermal water pools due to need to use untreated natural thermal water in such pools.

Conclusion

Total organic carbon (TOC) in mineral and thermal waters was measured. The method for TOC measurement was precise and accurate for the determination in mineral and thermal water. Some outdoor pools' water quality could be problematic, due to untreated water intake directly from the natural underground springs.

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SIMULTANEOUS/SELECTIVE VOLATMMETRIC DETECTION OF DICLOFENAC AND TETRACYCLINE IN WATER ON GRAPHENE MODIFIED-BORON-DOPED DIAMOND ELECTRODE

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Abstract

The graphene oxide modified boron-doped diamond electrode (GO/BDD) was obtained electrochemically and tested by cyclic voltammetry (CV) to detect diclofenac (DCF) from antiinflammatory pharmaceuticals class and tetracycline (TC) from antibiotics one, which belongs to emerging pollutants from water. Graphene reduced electrochemically from its oxide showed enhanced sensitivity in determining individual and selective or simultaneous of DCF and TC. The cyclic voltammetry method - based protocol for selective/simultaneous detection of DCF and TC was developed in this study using a commercial boron-doped diamond (BDD) electrode modified with graphene oxide (GO).

1. Introduction

Emerging pollutants (EPs) also known as emerging organic contaminants are defined as synthetic and natural substances, which belong to the category of a wide range of chemicals, personal care products and fire retardant compounds, surfactants, plasticizers, industrial additives, as well as pharmaceuticals, that are not commonly monitored in the environment but have the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects [1-2]. According to studies reported in the literature among the most common pharmaceutical groups detected in water, inflammatory / analgesics (paracetamol, acetylsalicylic acid, ibuprofen and diclofenac) and antibiotics (tetracyclines, macrolides, penicillins) are included [3]. Based on the structures of phenylbutazone, indomethacin and mefenamic acid, diclofenac, a derivative of phenylacetic acid, is the most widely used nonsteroidal anti-inflammatory drug (NSAID) in first-line therapy for pain, acute and chronic inflammation, arthritis, musculoskeletal, dermatomyositis, osteoarthritis, dental pain and other connective tissue systemic diseases [4, 5]. In order to obtain more information on the effects and occurrence of DCF on the environment, the European Union has decided to include temporary DCF in the First Watch List of the Water Framework Directive (EU 2015/495, European Commission) [6]. Whith a broad spectrum of activity against many grampositive and gram-negative bacteria produced by the Streptomyces genus of Actinobacteria, tetracycline is one of the many types of antibiotics drugs used to treat many different bacterial infections of the skin, intestines, respiratory tract, and other body systems, used for the treatment of bacterial infections such as syphilis, pneumonia, tuberculosis and plague among others [7-9]. In recent years, due to obtaining high sensitivities and selectivity in the detection of emerging pollutants by applying the electrochemical method and selecting the suitable electrode material, the development of electrochemical detection is of particular interest. As reported in the literature, electrochemical analysis brings considerable advantages compared to classical analytical techniques (HPLC, GC-MS) such as simplicity, shorter analysis time and reduced costs, offering improved performance in terms of reproducibility, sensitivity and detection limit compared to HPLC [10]. Through the proper use of electrode material, electroanalytical methods continue to be updated over time as required, developing higly sensitive detection protocols that are reproducible, reliable, cost-effective, and, with a high level of accuracy and precision. [11]. An electrode recognized for its remarkable properties with very good results in electrochemical detection applications is the commercial diamond electrode doped with boron. However, for better improvement of its electrocatalytic effect and the electroactive surface, graphene oxide is been reported to be suitable for modifying the electrode surface to get the desired performance (reproducibility, low background current, potentially large window and stability) [12–15]. Taking into account the results reported in the literature regarding the detection of DCF and TC on various electrodes, in this study, the modified electrode (GR/BDD) obtained by electrochemical reduction with graphene oxide was tested to detect individually and simultaneously/selectively DCF and TC from aqueous solutions.

2. Experimental

An Autolab potentiostat /galvanostat PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands) was used for performing the electrochemical experiments using with a classical three electrode cell. A platinum counter-electrode, a saturated calomel reference electrode (SCE) and the working boron-doped diamond (BDD) commercial electrode modified with graphene oxide (GO). The BDD commercial electrode provided by Metrohm (Herisau, Switzerland). Electrochemical deposition process with graphene oxide (GO) on the BDD electrode surface occurred at the potential of -1.50 V for 120 s by chronoamperometry (CA) technique, using a suspension of 4 mg/ml GO dispersed in water, when so-called GR/BDD electrode was obtained. The target analyte, diclofenac (DCF), was purchased from Merck and tetracycline (TC), was provided by Antibiotics (Romania). 0.1 M Na₂SO₄ was used as a supporting electrolyte in the electrodetection experiments. The electrochemical methods used in this research study were chronoamperometry (CA) and cyclic voltammetry (CV).

3. Results and discussion

3.1. Cyclic voltammetry (CV)

3.1.1. Individual detection of diclofenac (DCF) by CV

The commercial BDD electrode modified with graphene oxide (GO) reduced electrochemically named GR /BDD electrode was tested by cyclic voltammetry (CV) in the presence of various DCF concentrations and the series of cyclic voltamograms are presented in the Fig. 1. It can be noticed that the oxidation process started very early in comparison with unmodified BDD electrode (the results are not shnown here) at about -0.110 V / SCE, and two oxidation steps were identified, the second started at +0.540 V/SCE. Also, during reverse branch, two cathodic peaks occurred, the first at the potential of +0.150 V and the second at the potential of -0.140V suggesting quasi-reversible oxidation processes. A good linearity anodic and cathodic current vs. diclofenac concentrations was obtained for both regions with correlation coefficient of 0.993 and 0.998 (the obtained sensitivities were 0.021, 0.013, 0.014 and 0.025 $\mu A \cdot \mu M^{-1}$, respectively).



Figure 1. (a) Cyclic voltammograms recorded on GR/BDD electrode in 0.1 M Na₂SO₄ supporting electrolyte (curve 1) in the presence of 2–16 μ M DCF (curves 2–9), at a potential scan rate of 0.05 V·s⁻¹ in a potential range: -0.5 to +1V/SCE; (b) Calibration plot of the current vs. DCF concentration of the CVs recorded at E₁ = -0.110 V/SCE, E₂ = +0.540 V/SCE, E₃ = +0.150 V/SCE and E₄ = -0.140 V/SCE.

3.1.2. Individual detection of tetracycline (TC) by CV

The electrochemical behaviour of TC on GR/BDD electrode was studied by CV and the results are presented in Fig. 2. By testing the GR/BDD electrode in the individual detection of tetracycline, the oxidation process of TC starting later vs DCF, the first potential value of +0.530V/SCE, and the second at +0.822 V/SCE. Also, the sensitivities of 0.092 $\mu A \cdot \mu M^{-1}$ and 0.270 $\mu A \cdot \mu M^{-1}$ was obtained for TC detection at the potential values of +0.572 V/SCE and +0.822 V/SCE.



Figure 2. (a) Cyclic voltammograms recorded on GR/BDD electrode in 0.1 M Na₂SO₄ supporting electrolyte (curve 1) in the presence of 2–16 μ M TC (curves 2–9), at a potential scan rate of 0.05 V·s⁻¹ in a potential range: -0.5 to +1 V/SCE; (b) Calibration plot of the current vs. TC concentration of the CVs recorded at E₁ = +0.572 V/SCE and E₂ = +0.822 V/SCE.

3.1.3 Simultaneous detection of DCF and TC by CV

Considering the different behaviour of both target analytes, DCF and TC, related to the potential values for their oxidation, the simultaneous or selective determination can be developed by simple CV by selection of the potential range. For the whole potential range from -0.5 to +1 V/SCE, the simultaneous detection can be achieved, at about -0.110 V/SCE for DCF and at the positive potential values of +0.530 and respective, +0.822 V/SCE for TC.



(b) (c) Figure 3. (a) Cyclic voltammograms recorded on GO/BDD electrode in 0.1 M Na₂SO₄ supporting electrolyte (curve 1) in the presence of of 2–16 μ M DCF (curves 2–8), and of 2–10 μ M TC (curves 9–14) in a potential range: -0.5 to +1 V/SCE; (b) Calibration plots of the current vs. DCF concentration of the CVs recorded at E₁ = -0.110 V/SCE E₂ = +0.540 V/SCE, E₃ = +0.150 V/SCE and E₄ = -0.130 V/SCE; (c) Calibration plot of the current vs. TC concentration of the CVs recorded at E₁ = +0.530 V/SCE and E₂ = +0.822 V/SCE.

Table 1: Electroanalytical parameters obtained for individual and simultaneous detection of diclofenac and tetracycline on GR/BDD electrode using CV technique.

Туре	Analyte	E / V vs.	Sens.	R ²	RSD a	LOD b	LOQ °
		SCE	(μΑ/μΜ)		(%)	(µM)	(µM)
		p.a0.110	0.021	0.993	0.745	0.359	1.19
	DCE	p.a.+0.540	0.013	0.993	0.735	1.25	4.28
Individual	DCF	p.c.+0.150	0.014	0.993	1.12	0.934	3.11
marviauai		p.c0.140	0.025	0.998	0.780	0.870	2.88
	TC	p.a.+0.572	0.092	0.998	1.99	0.43	1.42
		p.a.+0.822	0.270	0.999	1.58	0.240	0.800
Simultaneous	DCE	p.a0.110	0.022	0.993	1.19	0.55	1.83
		p.a.+0.540	0.012	0.991	0.70	1.32	4.41
	DCI	p.c.+0.150	0.013	0.990	1.11	1.00	3.35
		p.c0.130	0.024	0.998	0.746	0.850	2.84
	тс	p.a.+0.530	0.095	0.993	1.39	0.411	1.37
	IC	p.a.+0.822	0.375	0.982	1.57	0.200	0.658

p.a.- anodic peak; p.c.- cathodic peak; a Relative standard deviation; b The limit of detection; c The limit of quantitation.

The results obtained on GR/ BDD electrode for individual and simultaneous detection of diclofenac and tetracycline by employing cyclic voltammetry technique are gathered in Table 1. The detection experiments for diclofenac detection were conducted in the presence of tetracycline, because the signal for diclofenac detection is lower. The individual and simultaneous detection of the target analytes on the GR / BDD electrode was achieved, the best performances in relation with the sensitivity, the limit of detection were achieved for the tetracycline detection, which can be selectively detected in the presence of DCF at more positive potential values. Also, DCF can be selectively detected at negative potential value, which is possible due to the presence of graphene.

Conclusion

Based on the above-presented results it can be concluded that GR/BDD modified electrode is very promising for the simulataneous/selective detection of diclofenac and tetracycline in water. The best performances in relation with the limit of detection and limit of quantification was achieved for TC detection.

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CHEMICAL AND GRANULOMETRIC PARAMETERS OF AGRICULTURAL SOIL IN THE SURČIN AREA - REPUBLIC OF SERBIA

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Abstract

In the period from June to October 2019, tests of chemical properties and granulometric composition of 280 composite soil samples were conducted in the area of Petrovčić and Progar, *Surčin Municipality (Belgrade, Serbia)*, to a depth of 30 cm. The presence of total sand, clay and silt fractions in the analyzed soil samples indicates a relatively uniform granulometric composition (textural class of light clays), but with unfavorable ratio of total sand/clay fractions, in which the clay fraction prevails. The largest number of soil samples has strongly acidic, medium and acidic values of substitution and active acidity, are carbonate-free, while the values of electrical conductivity are very low and indicate the complete absence of salinity in the surface soil horizon/layer. The largest number of soil samples is moderately provided with total nitrogen and humus, with very low, low and medium content of easily available phosphorus, as well as medium (CM Petrovčić) to high and medium provided with easily available potassium (CM Progar). The obtained results indicate that the studied soils of Petrovčić and Progar are suitable for intensive field production. However, low pH values indicate an adequate application of limestone and fertilizers, as well as measures such as undermining and drainage.

Introduction

The municipality of Surčin is the seventeenth municipality of the city of Belgrade (Republic of Serbia). It is located northwest of Belgrade and covers an area of 288 km². The municipality of Surčin is mostly characterized by agricultural-processing sector, and then by trade and service activities. The agricultural soil of Surčin covers the territory of two thirds of the total area of the municipality, about 198.16 km² [1].

According to Janošević [2], the share of agricultural soil in the municipality of Surčin in 2012 was 20142.5 ha, which is 72.6%. From a pedological point of view, data on the percentage share of certain types of soil indicate that the most common types of soil are fluvisols, as well as saline soils, humogleys and chernozems, which belong to the group of climatogenic (natural) soils, in which formation the climate had a decisive influence. The quality of these soils is very different. The most fertile areas are in Surčin around the airport, while the lower classes are in the settlements of Boljevci, Bečmen, Progar and Petrovčić. The agricultural soil area of the territory of Surčin for the studied areas CM Petrovčić and CM Progar in 2012 was 4091.6 ha. These soils mainly consist of arable soils intended for field production (Table 1).

During the field survey of studied areas it was observed that certain soils, intended for agricultural production, are marginal, in the sense that they have their limitations and are neglected (weedy) [3]. Thus, the position of Surčin itself, which gravitates to the capital city [1], requires the need to try to enable each surface and turn it into a production area.

In addition, one of the main causes of soil degradation in suburban settlements in the municipality of Surčin is the inappropriate application of agro-technical measures (mainly mineral fertilizers and plant protection products). However, the results of previous soil quality studies showed that the pesticides and heavy metals values are below the maximum allowable concentrations [2].

The mixed agriculture market of Surčin is characterized by a relatively high share of large agricultural organizations in the structure of arable soil use, low level of labor investment, high investment of capital, machinery and fertilizers, and high productivity, with a predominance of annual plants [4, 5].

Agricultural soil (ha)		Cadastral m	Total	
		Progar	Petrovčić	Total
	(1) Fields	2277.20	1548.96	3826.17
	(2) Gardens	0.00	0.00	0.00
Arabla	(3) Orchards	6.88	2.02	8.90
Arable	(4) Vineyards	3.87	0.63	4.50
	(5) Meadows	28.39	41.02	69.40
	(6) Total (1+2+3+4+5)	2316.35	1592.63	3908.97
	(7) Pastures	51.64	0.99	52.63
Non arabla	(8) Reeds, wetlands	43.84	5.69	49.53
Non-arable	(9) Other soils	14.02	66.45	80.47
	(10) Total (7+8+9)	109.50	73.13	182.63
-	Total (6+10)	2425.85	1665.75	4091.60

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Table 1. Agricultural soil area of Surcin for the studied territ	ories ir	n 2012 2	

Based on the mentioned natural conditions and problems of agricultural soil in the municipality of Surčin, the aim of the paper is to examine the condition of these soils through analysis of chemical and physical (granulometric composition) properties, with assessment of growing the crops that would give satisfactory yields.

Experimental

The trial was conducted in the area of Petrovčić and Progar, *Surčin Municipality*, City of Belgrade, Republic of Serbia (grid reference: 44°47' N, 20°16' E). Based on the available satellite images and the pedological map of Institute of Soil Science, scale R=1:50.000, the exact places of observations where sampling was performed were located.

Field work included taking composite soil samples with a probe at pre-determined locations in a disturbed state from a depth of 0-30 cm, according to the instructions, which refer to standard sampling methods [6, 7]. The total number of parcels within the municipality of Surčin, on which a total of 280 soil samples were taken during July and August 2019, was 49 (30 parcels belong to the CM Progar with 155 soil samples, 19 parcels belong to the CM Petrovčić with 125 soil samples). The total study area was 1674.84 ha. Figure 1 shows the position of the sampling point in the area of the Surčin Municipality for CM Progar and CM Petrovčić.

Climate data for a series of 16 observation years (period 2003-2018) were taken from the available meteorological yearbooks of the Republic Hydrometeorological Institute of the Republic of Serbia [8], and processed graphically. Data from the meteorological station Surčin (location: 44°49' N, 20°17' E), were used.

The relation between wet and dry periods during 2003-2018 is presented using climate diagram (Figure 2). Diagram shows that the dry period occurs from the end of June and lasts until the last decade of September. This precipitation regime does not positively affect either vegetable or fruit production. Such conditions are most favorable for crop production, since in the period sensitive to drought there is enough precipitation (June), but it is less suitable for winter crops, especially cereals, because the deficit occurs in the period when the plant is sensitive to drought.

The observation of the soil included an adequate sampling from the depth of 0-30 cm and preparation, where the soil samples were air-and, crushed and passed through a sieve with a diameter of $\leq 2 \text{ mm}$ [9], followed by granulometric composition and chemical analyses.

The share of clay, sand and silt fractions in soil (granulometric comosition, respectively), was analyzed by determination of particle size distribution in mineral soil material, using a standardized

method by sieving and sedimentation [10], after which the textural soil class was determined using the International Union of Soil Science (IUSS) texture triangle [11].



Figure 1. Location of soil sampling points

Figure 2. Temperatures and precipitations developments for 16 years of observation (2003-2018) for the studied locality

90 80

70

60

50

40

30

20

10

XI XII

Precipitation sum (mm)

The following chemical parameters were analyzed: soil acidity (pH in H₂O and 1M KCl, v/v: soil:H₂O=1:5, soil:1M KCl=1:5), potentiometrically, using glass electrode [12]; calcium carbonate (CaCO₃), using standard method [13], volumetric; electroconductivity (EC), according to documented method [14], conductometric; available phosphorus (P) and potassium (K), by AL-method according to Egnér-Riehm [15], where K was determined by flame emission photometry and P by spectrophotometer after color development with ammonium molybdate and stannous chloride; total nitrogen content (N), by dry combustion using elemental CNS analyzer Vario EL III [16]; humus content, using Kotzman method [14]. The results are presented in tables and graphs using Microsoft Office Excel 2007 statistical and mathematical program. The following values were calculated: mean (AVR), minimum (MIN), maximum (MAX), distribution and frequency. GIS software was used as a platform for geostatistical data analysis in spatial data processing.

Results and discussion

Table 2 and Figure 3 display the values of soil granulometric and chemical parameters, texture class and soil distribution in Petrovčić (125 samples) and Progar (155 samples).

The presence of total sand, clay and silt fractions in the analyzed soil samples indicates a relatively uniform textural composition. 94-98% of the examined soil samples belong to the textural class of light clays that have an unfavorable ratio of total sand/clay fractions, in which the clay fraction prevails. Since clayey soils retain water, it is often that waterlogging occurs on fairly flat terrain, which is the case in present study. This indicate an adequate application of measures such as undermining and drainage [5]. According to the values of substitution (pH in H₂O) and active (pH in 1MKCl) acidity, the largest number of samples has a strongly acid (<4.5) to acid (4.51-5.50) reaction. It was determined that almost all soil samples (122 and 143) are without CaCO₃ (92-98%) and are below the detection levels. This is completely according to the obtained pH values found previously, where low pH values indicate an adequate use of limestone [17]. Similarly, the values of EC are very low and indicate the complete absence of salinity of the surface soil horizon/layer. The supply of total N ranges from 0.12 to 0.20% in 80-85% of samples, and in 2-4% of samples the values of this parameter are from 0.05 to 0.12%.

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Cadastral municipality (CM)	Petrovčić (125 samples) Progar (155 samples)			ples)				
Textural class*	LC, 97%; SC, 2%; SCL, 1%			LC, 97%; SC, 2%; SCL, 1% LC, 94%; 1%; 5		LC, 97%; SC, 2%; SCL, 1% LC, 94%; HC, 2%; C 1%; SCL, 1%; S		L, 1%; SL, C, 1%
Granulometric and chemical parameters	AVR	MAX	MIN	AVR	MAX	MIN		
Sand (%), fraction >0.02 mm	27.8	35.4	12.7	26.7	39.1	14.6		
Clay (%), fraction <0.002 mm	35.9	44.7	22.2	36.9	46.5	13.0		
Silt (%), fraction 0.02-0.002 mm	36.2	55.5	25.4	36.5	57.7	26.7		
pH (1M KCl)	4.78	6.95	3.60	4.74	7.40	3.80		
pH (H ₂ O)	5.71	8.00	4.60	5.80	8.50	4.80		
$CaCO_3(\%)$	0.50	0.65	0.43	5.35	14.96	0.65		
EC (µS cm)	54.50	366.00	21.20	50.10	175.80	17.60		
Available P (mg 100g ⁻¹)	4.81	121.80	0.11	9.60	58.55	0.11		
Available K (mg 100g ⁻¹)	20.33	109.50	8.06	25.92	119.54	9.35		
Total N (%)	0.18	0.41	0.11	0.15	0.28	0.09		
Humus (%)	2.74	4.59	1.44	2.68	6.23	1.35		

Table 2.	Granulometric	composition and	chemical	properties	of the studie	d area soil samples
		1		1 1		1

*LC-Light Clay; HC-Heavy Clay; CL-Clay Loam; SL-Silty Loam; SCL-Silty Clay Loam; SC-Silty Clay





The supply of humus in 97-98% of the samples is in the range of 1.50 to 4.00%, which is a property of slightly clayier soils [17]. The content of easily available P in 91% of samples (113 samples) from the area of Petrovčić is very low, while in the area of Progar 50% of samples (78 samples) have a very low, 18% (28 samples) - low and 16% (25 samples) - medium content of the tested element. The low phosphorus content may be a consequence of its immobilization by binding to aluminum and iron in acid soils, as well calcium in alkaline soils, but also of less fertilization with this element [17, 18]. In the area of Petrovčić, the largest number of samples (68 samples, 54%) is with medium content of easily available potassium, while 25% of samples (31 samples) have high content. Soil samples, taken in the area of Progar, are richer in potassium, so the largest number of samples is highly provided (67 samples, 43%) and medium provided (58 samples, 37%) with available potassium.

Conclusion

Results of granulometric and chemical parameters of the soils and their distribution indicate that the examined soils of Petrovčić and Progar, *Surčin Municipality*, are mostly suitable for intensive field production, especially wheat, corn and sunflower. Nevertheless, low pH, mostly medium and low values of other chemical parameters and clayey soils indicate an adequate application of limestone, fertilizers, as well as measures such as undermining and drainage.

Acknowledgements

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MICROENCAPSULATION OF SOME GLUCOSINOLATES FROM BRASSICA FAMILY EXTRACTS BY β -CYCLODEXTRIN

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Microencapsulation is defined as a process of packaging or the presence of protective active agents within a carrier material to improve delivery, maintaining integrity or increasing the solubility of active compounds into pharmaceutical or food products [1]. Our experiments tried to microencapsulate the glucosinolates in β -cyclodextrin from 70% ethanolic-water extracts of indigenous vegetables of *Brassica* family [2]: cabbage, acclimatized broccoli, cauliflower, black radish, and kohlrabi in order to protect the glucosinolates against hydrolysis to isocyanates or isothiocyanates and in order to increase their thermal resistance (e.g., frying). The microencapsulation process occurs by direct contacting of the 70% ethanolic-water extracts with β -cyclodextrin, under stirring, during 18-24 h, at 50-52°C and then evaporation of remaining solvent. The obtained powders were characterized by FTIR, RX, SEM images and spectral absorption/reflectance in order to demonstrated the encapsulation process (figure 1).



Figure 1. Spectral reflectance curves of complex β Cy-natural extracts

All studied powder complexes show smaller reflectance than β -Cy (λ =367-376 nm) and this can suggest the encapsulated process. All effectuated analyses sustain the microencapsulated process.

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COMBINING UAV-BASED ZONE SPRAYING AND VRA TECHNOLOGY TO ACHIEVE A 50% CHEMICAL DECREASE FOR EU'S GREEN DEAL

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Abstract

Agricultural-origined polluting is one of the greatest problems of humanity nowadays, as great as producing food for every people in the world. We wanted to prove the relevance of new UAV based technologies in agriculture, and their efficiency in the decreasement possibilities in chemical usage. Our research shows that the European Green Deal could be accessible (or available) with precision farming combined with drone technologies. Introduction

Agriculture is one of the most polluting areas of human activity, because of using fertilizers, insecticides, pesticides and herbicides. However, almost 8 billion people have to eat. Humanity has just one possibility to solve the problems of 21th century: with new technologies. If we want to achieve the agricultural chemical decrease goals of the European Green Deal by the year 2030 (50%), we have to develop and use new technologies, for example agricultural drones in pest and noxious weed control. Advantages of drones (UAV) are the following: no treading, no compression and most importantly approximately -30% need of chemical dosage can be achieved (diploma thesis, in press)! Using agricultural drones is legal and widespread in many countries of the world, but in Hungary only the experimental use is accepted yet. Cirsium arvense and other "injurious weeds" are causing problems in certain regions of the world (Europe, Canada, New Zealand etc.). Cirsium arvense is an erect perennial rhizomatous thistle. It is usually 0.5 - 1.0 m tall, distinguished from all other thistles by dense clonal growth, creeping horizontal lateral roots and male and female flowers are on separate plants. Cirsium leaves glabrous below with many marginal spines.^[1] The traditional method of chemical weed contol (for perennial weeds like Cirsium arvense, Convulvulus arvensis, Asclepias syriaca...) is a "blanket application" on the field with glyphosate (or other total herbicide) by tractor with 5 l/ha dosage in solution 300 l/ha (with 4 l/ha Nitrosol (Liquid nitrogen (30%) fertilizer) and 0,01% Trend 90), which means the whole parcel plot is being sprayed, no matter if there is any weed or not.

Our research is closely related to the trend of the European Union: experimenting necessary doses of the allowed chemicals to the new treatment technologies for drones. In this research we were focusing on Cirsium arvense, a geophyton weed which has an overwinter body in the soil, so defence is very difficult against it. The best way to eliminate geophytons is the treatment with absorbed chemicals, such as glyphosate, in autumn or spring before seeding.^[2]

Experimental

An 18 ha field was treated with herbicide (near an untreated control field), the previous crop was cereal. Glyphosate (N-Phosphonomethyl-glycine) products are one of the most widely used weed killers worldwide in farms and in home gardens, it is used in 1600 t/year in Hungary. It's licence will expire in 2025 December, but there are no other alternative herbicides yet. DJI Phantom 4 Multispectral (P4M) drone was used for orthoimagery. This drone has a head sensor with 6 cameras (Red, Green, Blue, RedEdge, NIR, RGB) and a built-in sunlight sensor for accurate results. The flights and the routes can be planned, and operated by the DJI GS Pro Ipad

app for drone operations (free software, <u>https://apps.apple.com/us/app/dji-gs-pro/id1183717144</u>). DJI TERRA software was used for analyzing, processing, visualizing, and planning spraying works for the AGRAS agricultural drones. DJI AGRAS T20 agricultural drone (container volume 20 liters, route width 6 meters on 2,5-3m route altitude) was applied for the spraying of herbicide.

The experiment was planned for a 18 ha treated parcel and an untreated, control parcel. If we had used the traditional method we would have needed 18 ha x 5 1/ha = 901 of glyphosate. Earlier experiments showed that the traditional spraying with tractor has approximately 40-60% solution waste (on the ground). Spraying with drones also have waste, but significantly less than the traditional way (10%, in press). Our main goal was to achieve the minimal possible environmental impact with the appropriate herbicide effect.

In the experiment, our zone spraying method was combined with DJI's Various Rate Application (VRA) technology, which is based on the NDVI values collected by the P4M. The resolution of the generated prescription map for VRA was 6x6 meters.

Three different dosages of glyphosate solution were sprayed, depending on the infection rate of the weed spots (weak, moderate and strong infection). 8 l/ha solution was sprayed to the weakly infected areas (NDVI \leq 0,1), 10 l/ha to the moderately infected areas (0,1 < NDVI < 0,2), and 12 l/ha solution was applied to the strongly infected parts (NDVI \geq 0,2). The base solution was 10 l/ha containing 3,5 l of glyphosate.



Figure 1. NDVI picture of the different infected areas (left side: control, right side: before treatment).

The treatment took 2.5 hours to finish the VRA zone spraying for our Beta Version Agras T20 drone, which means 7.2 hectars/hour with 6 m route width, on 2,5-3 m route altitude. The weather on the day of the experiment was: 18 °C and 5 km/h wind.



Figure 2. Prescription map of the examined field.



Figure 3. VRA zone spraying (green line: spraying, white line: traveling without spraying)

Results and discussion

We have sprayed Cirsium arvense and other weeds on the 21th of September 2021 and the final efficiency of the application and the choosen dosages were checked on the 12th of October (21 days). The drone used a total ammount of 83 liters of solution, containing 29 liters of glyphosate. The traditional blanket application would have used a total of 90 liters of glyphosate on the 18 hectares, so in our experiment we managed to achieve a 67,78% of chemical saving. We assumed that the higher specific surface area, which is caused by the the smaller droplet sizes of the solution, and the better coverage due to rotor wind were consented to the success. The efficiency of the treatment was the same as traditional methods. Treated areas were monitored manually and by the multispectral drone once a week.



Figure 4. NDVI values of Cirsium arvense weed spots



Figure 5. Treated parcel before and after spraying (21 days).

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IDENTIFICATION OF UNKNOWN FILAMENTOUS FUNGI FROM WILLOW WOOD AND SORGHUM CHIPS

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Abstract

Molecular biological methods are generally applied in the identification processes of microorganisms. We aimed to isolate numerous cellulolytic filamentous fungi strains from willow wood and sorghum chips, and attempted to identify them with polymerase chain reaction (PCR).

Modified Czapek-Dox medium was used with the addition of microcrystalline cellulose and carboxymethyl cellulose (CMC) as a source of carbon, in order to isolate cellulolytic filamentous fungi strains. Through sequence-based identification, representatives of the genera *Trichoderma, Aspergillus* and *Fusarium* were identified.

Introduction

Filamentous fungi create a diverse and often well-described group in the kingdom Fungi, although a high number of unidentified species exists. The classical, morphology-based identification of filamentous fungi often proves to be inadequate due to the morphological similarities among the different species. Therefore, molecular biological methods are usually required along with macro- and micromorphological studies in order to identify these diverse microorganisms [1].

Filamentous fungi have been known as proficient colonizers of lignocellulosic plant tissues and have been recognized as producers of exceptionally rich and diverse hydrolytic enzymes [2], [3]. This characteristic makes them valuable candidates for the biological pre-treatment of substrates before anaerobic digestion (AD). AD is a four-stage process including hydrolysis, acidogenesis, acetogenesis and methanogenesis. In this process, a wide range of organic wastes can be degraded, and a carbon-neutral, renewable energy carrier, biogas is produced [4]. Pre-treatment before AD can enhance not only the biogas yields during AD but the amount of degraded organic materials as well. Physical and chemical pre-treatments can be applied, biological pre-treatments are the most environmentally friendly methods.

Experimental

Willow wood and sorghum chips colonized by filamentous fungi were selected randomly.

Microcrystalline cellulose and carboxymethyl cellulose (Merck, Darmstadt, Germany) were applied respectively as a source of carbon in modified Czapek-Dox medium during isolation (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium130.pdf), in order to isolate cellulolytic filamentous fungi. The medium contained 100 μ g/ml of antibiotics (ampicillin and streptomycin, respectively) to prevent bacterial growth.

Stereomicroscopic images were taken with a Nikon SMZ745T stereo microscope (Fig. 1-4.). After several re-plating on the cellulose-rich media, pure isolates were obtained. Pure cultures



Figure 1-4.

were maintained on Potato Dextrose Agar (Sigma-Aldrich, St. Louis, USA). Plates were incubated at 25°C during both isolation and maintenance.

Micromorphology of the pure isolates was examined using Lactophenol Cotton Blue stain, and images were taken with an Olympus BX53 microscope.

The identification process included various molecular biological methods such as DNA isolation, polymerase chain reaction (PCR), agarose gel electrophoresis, DNA purification, and DNA sequencing.

DNA isolation

Pure filamentous fungi strains were inoculated into Potato Dextrose Broth (Sigma-Aldrich, St. Louis, USA), respectively. After 5 days of incubation at 25°C, DNA samples were prepared with the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, CA, USA).

Polymerase Chain Reaction

Isolated DNA samples were amplified in polymerase chain reactions (PCR). Three different sets of primers were applied in various reactions. In the table below, the circumstances of each PCR step and the primers are described (Table 1.).

Name of the primer	CMD5/6 [5]	RPB2 [6]	FU2/3 [7]
Forward primer sequence	5' - CCGAGTACAAGGAGGCCT TC - 3'	5'- GAYGAYCGKGAYCAYT TCGG-3'	5' - GGTCGCCGTAAGAGGGGT TGG - 3'
Reverse primer sequence5' - CCGATAGAGGTCATAACG TGG - 3'		5'- CCCATRGCYTGYTTRCC CAT-3'	5' - CGAGCCCGGTACCATGGA CG - 3'
1. First denaturation 95 °C - 5 min		95 °C - 3 min	94 °C - 3 min 30s
2. Denaturation	95 °C - 30 s	95 °C - 30 s	94 °C - 1 min
3. Annealing	56 °C - 30 s	56 °C - 30 s	60 °C - 30 s
4. Elongation	72 °C - 30 s	72 °C - 1 min 20 s	72 °C - 2 min
5. Final extension	72 °C - 2 min	72 °C - 3 min	72 °C - 5 min
Number of cycles (step 2-4)	35x	35x	35x

Table 1.

Agarose Gel Electrophoresis

After PCR, the amplicons were separated by their sizes using agarose gel electrophoresis. In this experiment TAE (Tris-Acetate-EDTA) buffer and 1% agarose gels were used, and the agarose gel electrophoresis lasted 35 min at 90V.

DNA extraction

Appropriately sized DNA fragments were cut from the agarose gel and extraction of the amplicons was carried out with the GeneJet Gel Extraction Kit (ThermoFisher Scientific, Waltham, MA, USA), following the manufacturer's protocol.

DNA sequencing

Samples were sequenced at the Molecular Genomics Centre of the Biological Research Centre (Szeged) on a 3500 Series Genetic Analyzer (Life Technologies, Carlsbad, CA, USA).

The DNA sequences were filtered and analysed using BLAST against the NCBI database.

Results and discussion

After the series of experiments, six strains of filamentous fungi were identified, belonging to the genera *Trichoderma, Aspergillus* and *Fusarium*.

Two strains proved to be *Trichoderma harzianum*. Identification was based on the segment of the calmodulin gene, which was amplified using the primers CMD5/6. The isolates showed 100.00% and 95.81% sequence similarity to deposited sequences, respectively, and were studied micromorphologically as described above (Fig. 5.).



Figure 5.

Aspergillus clavatus from the genus *Aspergillus* has also been identified. The *A. clavatus* isolate has shown 98.77% sequence similarity to deposited sequences, based on the amplicons of the second largest subunit of the nuclear DNA-dependent RNA polymerase II (with RPB2 primers). Macromorphological characteristics of *A. clavatus* support the result of molecular biological methods (Fig. 6.).

From the environmental samples, Fusarium solani and other representatives of genus Fusarium



Figure 6.

have been identified with the application of FU2/3 primers, which primers amplify a partial β -tubulin gene fragment. These fungi are known as pathogenic strains, therefore the samples were eliminated from further experiments.

Conclusions

Filamentous fungi have a key role in the deconstruction of the lignocellulosic biomass of various origins due to their remarkable enzyme synthesizing ability. The bioavailability of resistant plant cell wall biopolymers can be considerably improved by fungal pre-treatment, which makes the identification of cellulolytic filamentous fungi crucial for future applications. The identified fungal strains in the present study, belonging in the genera *Trichoderma* and *Aspergillus*, can serve as relevant candidates in enzyme activity assays, and in future experiments including fungal pre-treatment with the aim of enhancing biogas production.

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2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-AZO-PYRIDONE DYE: A POTENTIAL APPLICATION AS NEW GREEN-EMITTING FLUORESCENT PROBE

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Abstract

Molecular imaging is a relatively new research field, which has demonstrated great potential, especially in clinical oncology – from drug development to cancer early detection. The key of fluorescence imaging is the construction of fluorescent probe which is composed of two parts, the recognition groups to recognize cancer cells, and fluorophores to signal the recognition events. In this research, the structure of new fluorescent azo dye based on 2-oxo-1,2,3,4-tetrahydropyrimidin and 2-pyridone moieties has been reported. The absorption and emission properties of the investigated azo dye have been studied using UV-Vis and fluorescence spectroscopy. The obtained results suggest that studied dye meets the requirements for new green-emitting fluorescent probe, suitable for further application in biomedical researches.

Introduction

Fluorescent probes are one of the major driving force of the molecular imaging. They are the agents used to visualize, characterize and measure biomolecules and biological processes in living systems [1,2]. Fluorescent molecules absorb light of a specific wavelength and emit light of a longer wavelength. Emission variations of the bound fluorescent compound are indicators of changes in the conformation of biomolecules, providing a useful tool for tracking biological pathways [3]. For surface applications, such as detecting tumors on epithelial surface, lower wavelength (e.g., blue, green, yellow) emitters, with high quantum efficiency, may produce as good or better results compared to the NIR emitters [4]. In this research, structure of new fluorescent azo dye, and its absorption and emission properties have been reported. In order to design compound with fluorophores and pharmacophores, 2-oxo-1,2,3,4-tetrahydropyrimidin based diazonium salt has been coupled with 3-cyano-6-hydroxy-4-methyl-1-phenyl-2-pyridone, resulting in a new fluorescent azo dye. The structure, absorption and emission properties of the investigated azo compound have been studied using ATR-FTIR, NMR, UV-Vis and fluorescence spectroscopic measurements.

Experimental

Synthesis

The synthesis of reported azo dye has been in detail described in our published study [5]. In brief, 2-oxo-1,2,3,4-tetrahydropyrimidine-azo-pyridone dye (PHPD) was prepared within diazo-coupling reaction. Pyrimidine derivate, 4-(4-aminophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate was utilized as diazo component, and 3-cyano-6-hydroxy-
1-phenyl-2-pyridone was a coupling component (Fig. 1). The structure of the dye was confirmed by spectroscopic data [5]. The FTIR-ATR spectra were recorded using a NicoletTM iSTM 10 FT-IR Spectrometer (Thermo Fisher Scientific). The ¹H NMR and ¹³C NMR spectral measurements were performed on a Bruker Ascend 400 instrument (400 Hz and 100 MHz, respectively) in deuterated dimethylsulfoxide (DMSO-*d*₆).



Fig. 1. Synthesis of PHPD

Optical measurements

The optical measurements were conducted in four solvents of different features. The UV-Vis absorption spectra were recorded on Shimadzu 1700 spectrophotometer, at concentration 4×10^{-5} mol L⁻¹, while emission spectra were recorded, at the same concentration, on Shimadzu RF-1501 PC spectrofluorometer. All spectroscopic measurements were carried out at room temperature (25 °C).

Results and discussion

The synthesized azo compound is a dark orange powder of high purity, and it was obtained in a good yield (about 70%). The spectral data of PHPD suggest the existence of the hydrazone tautomeric form (Fig. 1) in the solid state, as well as, in the DMSO- d_6 solution. The ATR-FTIR measurements displayed that N–H stretching vibrations of the hydrazone group appear at 3248 cm⁻¹. The bands at 1678 cm⁻¹ and 1629 cm⁻¹ are ascribed to the vibrations of carbonyl groups. The ¹H NMR spectrum showed the signal of hydrazone N–H group at 14.64 ppm, and ¹³C NMR spectrum contained the signal at 161.11 ppm, confirming the existence of hydrazone tautomeric form [5].

The absorption and emission spectra were recorded in the range from 300 to 700 nm in following solvents: acetonitrile, DMSO, ethanol and chloroform (Fig. 2). From the presented optical spectra, it can be observed that used solvents had negligible effect on the position of absorption, as well as emission maxima. The obtained optical spectra suggest the existence of hydrazone tautomeric form in case of all used solvents. An intense band appearing in UV-Vis spectra, in the region of 370-550 nm, is ascribed to the intramolecular charge transfer (ICT) of the hydrazone tautomeric form [6]. The emission maxima of the investigated dye are in the region of 530 nm, and corresponding Stokes shifts are between 76 and 88 nm, indicating the recommendable properties for application as the potential new fluorescent probe.



Figure 2. The UV-Vis and fluorescent spectra of the investigated PHPD dye

In addition, the biocompatibility assay, conducted within our previous study, demonstrated the non-toxic effect of the the investigated potential fluorescent probe ($IC_{50} = 194.56 \pm 9.43 \mu M$) to the normal human fibroblast cell line (MRC-5), suggesting that studied fluorescent azo dye is suitable for further investigation related to its potential application in different *in vitro* and *in vivo* models [5].

Conclusion

In this work, structure and optical properties of new potential fluorescence probe based on 2oxo-1,2,3,4-tetrahydropyrimidine and 2-pyridone scaffolds have been presented. The FTIR-ATR and NMR analysis have shown that synthesized azo dye appeared in a solid state, as well as in the DMSO- d_6 solution, in hydrazone form. The absorption and emission spectra of the investigated dye were studied in solvents of different characteristics. The obtained absorption maxima were positioned in the region of 440 nm, while the emission maxima were in the green spectral region, with Stokes shifts ranking from 80 to 90 nm. Presented results may serve for further development of new green-emitting fluorescent probe and its potential application in fluorescence imaging.

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INCUBATION-BASED HYDROXYAPATITE SYNTHESIS METHOD USING SHELLS AS Ca²⁺ SOURCE

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Abstract

Hydroxyapatite, the well-known calcium phosphate, was obtained on the surface of shells fragments by partial conversion of the latter, which served both as template and also provided the calcium ions required for synthesis. Confirmation of hydroxyapatite formation was acquired by recording and analyzing X-ray diffraction patterns. The degree to which the phosphate ions were consumed during the incubation-based synthesis was determined using a phosphate minicolorimeter, and the results indicate that a nucleation process takes place in the first 4 hours.

Introduction

The unique osteoconductive, osteoinductive, osseointegrative, biodegradabile and corrosion protective properties of hydroxyapatite [1-5] make it one of the most widely used calcium phosphates in the biomedical field. An important quality possessed by this ceramic material is its ease of synthesis, which has led to the development of several methods for obtaining it, including the hydrothermal, solvothermal, homogeneous precipitation and electrochemical deposition techniques [6,7]. On the other side, depleted shells are an abundant renewable resource, which in some cases may represent an ecological problem [8]. The use of shells as Ca source for the synthesis of useful materials is one way to use this resource in a highly desired manner. Herein, we present an incubation-based method for obtaining the specified material that employs a significantly lower temperature than the ones used in the standard hydrothermal synthesis, for example. Furthermore, the calcium precursor is provided by the exoskeleton fragments of sea shells that also serve the role of hydroxyapatite deposition substrate. The synthesized product was characterized by X-ray diffraction and the results show the presence of hydroxyapatite. The phosphate ions consumption degree during the incubation experiments was determined as a measure of hydroxyapatite formation.

Experimental

Shells were collected from the Black Sea, washed repeatedly with distilled water and dried in the oven at 80 °C for 12 h. The fragments of shells used for synthesizing hydroxyapatite were selected by grinding and sieving, and the retained fraction is the one between 1 and 1.6 mm. The phosphate precursor was Na₂HPO₄ x 12H₂O (Reactivul București) and the calcium precursor was the CaCO₃ from the shells. Incubation experiments were performed with a Hygiena dry bath incubator and the shells together with the phosphate precursor were introduced in glass vials that were subsequently closed (Figure 1). The phosphate precursor helped to ensure the initial solution pH of 9.18. The Na₂HPO₄ x 12H₂O concentration in the solution was set so as to obtain a calcium carbonate conversion degree of maximum 5%. The effect of the time period on the reaction between the two precursors was monitored at 90 $^{\circ}$ C, and the experiments lasted between 4 and 112 hours.



Figure 1. Glass tube containing a sample of shells, fraction 1-1.6 mm, and the phosphorus precursor solution, before the experiment

Results and discussion

After the experiments ended, the shells samples were collected, washed and dried, and they were structurally characterized *via* X-ray diffraction. The obtained spectra were analyzed in order to determine their composition. The comparison of the diffraction maxima recorded on the samples and their theoretical positions (Figure 2) indicates that during the incubation period hydroxyapatite with low crystallinity was synthesized on the surface of the exoskeletons fragments.



Figure 2. X-ray diffraction spectra of samples obtained at 4 h, 30 h, 48 h, and 112 h. The spectrum at the bottom is a standard from the ICDD database, file no. 9-432

Accurate identification of the presence of hydroxyapatite is difficult, due to a number of factors: the low quantity, the presence of large amounts of aragonite and calcite with high crystallinity, as well as the overlapping of hydroxyapatite maxima positions with those corresponding to the other phases present in the sample.

In order to raise the degree of confidence regarding the formation of the desired product, the sample obtained in the incubator at 112 h was further calcined at 200 °C and 350 °C, and the spectra recorded on these samples are shown in Figure 3. With the increase in temperature, the improvement of the reflections corresponding to the hydroxyapatite crystalline structure can be observed, together with a clearer delimitation of the signals and an increase in their resolution.



Figure 3. X-ray diffraction spectra of: the sample obtained at 112 h, the same sample calcined at 200 °C and 350 °C, and (at the bottom) the standard hydroxyapatite spectrum from the ICDD database

The degree of phosphate ions consumption was determined as a measure of hydroxyapatite formation, by first finding out the level of phosphate ions for the precursor solution and then for the solutions collected after the completion of the experiments. In order to do this, a phosphate minicolorimeter (Hanna Instruments) and the standard method of the apparatus were used. A total of three determinations were made for each sample and their arithmetic mean was calculated. After the incubator synthesis at 90 °C, for different time periods, each sample was washed repeatedly with small fractions of distilled water. These fractions were collected and the obtained solution was filtered through a 0.22 μ m cellulose filter. Each resulted solution was used to fill a volumetric flask to its marking and subsequently, *via* successive dilutions, its concentration was brought in the measuring range of the apparatus. The degree of phosphate ions consumption obtained based on the performed determinations and calculations is presented in Table 1.

Table 1. Consumption degree of phosphate ions as a function of the time allocated for each experiment

Experiment duration (h)	4	30	64	112
Degree of consumption (%)	94.7	97	97.7	97.8

As can be seen, there was a high degree of consumption in all cases, but the results also show that in the first 4 incubation hours the phosphate ions concentration decreases to a much larger extent than it does after this time period. It is probable that a nucleation process takes place during these 4 hours in which most of the phosphate ions are consumed. Once this process comes to an end, the consumption rate diminishes substantially and keeps decreasing inversely proportional to the incubation time.

Conclusion

Hydroxyapatite was obtained on the surface of exoskeleton fragments of sea shells following an incubation-based synthesis method, in which the shells provided the Ca precursor. Shells also represent the substrate for the synthesis of composites with hydroxyapatite. X-ray diffraction structural analysis was used to confirm the presence of hydroxyapatite on the shells surface. The phosphate ions consumption degree during incubation was also determined and it indicates the existence of a nucleation process that takes place in the first 4 hours.

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PRELIMINARY WATER SPLITTING STUDIES ON Ag AND GRAPHITE MODIFIED POROUS STRUCTURES, AS SUCH OR DECORATED WITH Pt PARTICLES

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Abstract

Porous materials were obtained using polyether foam modified with Ag nanowires and graphite paste. Some of those modified porous structures were decorated with Pt particles *via* double step chronoamperometry. All porous materials were investigated in terms of their electrocatalytic activity for the oxygen and hydrogen evolution reactions (OER and HER) in alkaline medium, after they were inserted into supports made from sintered graphite (for the OER experiments) or Ag wire (for the HER experiments). Electrochemical stability tests were also performed. The results of the OER and HER experiments show that the most catalytically active porous structure is the one modified with graphite paste and Pt particles. Stability tests data show that the porous electrode based on this structure is very stable.

Introduction

As the global energy demand and environmental concerns increase, there is growing interest for the energy conversion and storage domain, in which the role of hydrogen production processes cannot be overlooked. Electrochemical water splitting is one way of obtaining this useful gas and it involves two half-cell reactions: the oxygen evolution reaction (OER) and the hydrogen evolution reaction (HER) [1].

In practice, in the presence of certain materials the energy required for the occurring of the two half-cell reactions decreases and, as a result, they take place at electrochemical potential values that are closer to the theoretical ones than those at which they would have occurred in the absence of the materials. They possess electrocatalytic properties for the OER and/or HER, and in the last few years several such structures with high catalytic activity have been reported in the scientific literature [2-6].

Herein, we present the preliminary results obtained using porous structures modified with Ag or graphite, as such or further decorated with Pt particles, to catalyze the OER and HER half-cell reactions in strong alkaline medium.

Experimental

In order to manufacture porous electrodes, polyether foam cylinders ($\phi = 12$ mm, height = 5 mm) were obtained from a polyether foam block (Jiejie, Beijing). After a cleaning stage they were covered either with Ag nanowires synthesized using a modified polyol method [7] in which the polyol was replaced with ethylene glycol (Honeywell) or with graphite paste obtained from graphite flakes (325 mesh particle size, Sigma Aldrich) that were ground using the ball mill for 12 hours. Some of the resulted modified porous structures were decorated electrochemically with Pt particles using two consecutive potential steps for different time intervals (E₁ = -0.5 V and E₂ = 0 V vs. SCE; t₁ = 5 s and t₂ = 60 s), in electrolyte solution obtained by adding 5 mM H₂(PtCl₆) x 6H₂O solution (5 mL) in 0.5 M H₂SO₄ solution (20 mL).

The reagents were purchased from Sigma Aldrich. For the HER experiments the porous structures were inserted into a silver support made from Ag wire processed in the form of a spiral (999 – 1,5 mm x 50 cm, Artkimia), while for the OER experiments a sintered graphite support was manufactured and coated with polyurethane resin, except for the area where the porous material was placed (Figure 1). These supports and the ensembles (modified porous structures introduced into the supports) are the electrodes investigated in the study, coded as specified in Table 1.



Figure 1. Ag wire support, $\phi_{int} = 11 \text{ mm}$ and thickness = 3 mm (a); Ag wire support with Ag modified porous material (b); Graphite support, $\phi_{int} = 11 \text{ mm}$ and thickness = 5 mm (c); Graphite support with graphite modified porous material (d)

Table 1	. The codes for the	studied support type electrodes and modified porous electrodes	;

Electrode code	Details about the studied electrode					
S _G	The graphite support					
S _G G	The graphite support with graphite modified porous					
	structure					
S _G GP	The graphite support with graphite modified porous					
	structure decorated with Pt particles					
SA	The Ag support					
S _A G	The Ag support with graphite modified porous structure					
S _A GP	The Ag support with graphite modified porous structure					
	decorated with Pt particles					
S _A A	The Ag support with Ag modified porous structure					
S _A AP	The Ag support with Ag modified porous structure					
	decorated with Pt particles					

The electrochemical experiments were performed using a glass cell equipped with three electrodes connected to a Voltalab PGZ 402 potentiostat (Radiometer Analytical). The counter electrode was a Pt plate ($S_{gcom} = 0.8 \text{ cm}^2$), the Ag/AgCl (sat. KCl) electrode was used as reference and each of the electrodes presented in Table 1 was used as working electrode. The measured potentials *vs*. the specified reference electrode were subsequently converted to the Reversible Hydrogen Electrode (RHE) using the standard equation [8]. The O₂ and H₂ evolution overpotentials were determined using equations (1) and (2). The electrolyte solution was 1M KOH (the potassium hydroxide was procured from Merck), the employed methods were linear and cyclic voltammetry, the recorded curves were iR-corrected using the Ohmic Drop Comp. option from the VoltaMaster 4 potentiostat software, the scan rate (v) was 5 mV/s, and prior to the HER experiments the electrolyte solution was deoxygenated by bubbling nitrogen 4.6 for 10 min.

$\eta_{O2} = E_{RHE} - 1,23$	(1)
$\eta_{H2} = E_{RHE} $	(2)

Where, E_{RHE} = the RHE potential [V], η_{O2} and η_{H2} = the O_2 and H_2 evolution overpotentials [V].

Results and discussion

OER experiments. The linear voltammograms recorded on the S_G , S_GG and S_GGP electrodes are presented in Figure 2. At low current density values it is observed that an anodic signal is present on the polarization curve obtained using the S_GGP electrode. This signal corresponds to an oxidation process taking place simultaneously with the OER and makes it difficult to identify the overpotential values at which the oxygen evolution takes place in the potential range in which it is evidenced. The feature is absent from the other voltammograms and at high current density values there are no other processes taking place, on any of the traced curves, that overlap with the OER. However, despite the signal, it is clear that the electrocatalytic activity of S_GGP for the oxygen evolution reaction is significantly higher than that of the other two electrodes. The experimental results indicate that Pt particles decoration of the graphite modified porous structures has the effect of decreasing the O₂ evolution overpotential.



Figure 2. Linear voltammograms recorded on the S_G , S_GG and S_GGP electrodes, in 1M KOH solution, at v = 5 mV/s

The three electrodes were further characterized in terms of their electrochemical stability using cyclic voltammetry. Figure 3 shows the 1^{st} and 10^{th} cycles obtained for each electrode, and it can be observed that the graphite support has the lowest electrochemical stability. This is because the maximum value of the current density reached during the O_2 evolution when the first cycle is recorded changes significantly by the time the 10^{th} cycle is obtained. The other two electrodes are much more stable and this means that the introduction of the porous structures into the graphite support leads to an increase in its stability.



Figure 3. Cyclic voltammograms recorded on the $S_G(a)$, $S_GG(b)$ and $S_GGP(c)$ electrodes, in 1M KOH solution, at v = 5 mV/s

HER experiments. The linear voltammetry curves shown in Figure 4a reveal that, out of the S_A , S_AG and S_AGP electrodes, the most catalytically active for the HER is S_AGP , both at low current

density values, as well as at high ones. However, regarding the very high HER electrocatalytic activity of the S_AGP electrode, it is important to note that the graphite modified porous structures obtained and investigated in the present study display some disadvantages. Firstly, the paste covering them is not highly adherent to the polyether foam substrate, and secondly, after the immersion of the porous structures into the electrolyte solution and during H₂ release it is strongly recommended to use a peristaltic pump to ensure the circulation of the solution through the pores for the removal of trapped gas bubbles. Addressing these issues will be the subject of future studies.

Figure 4b presents the curves obtained for the S_A , S_AA and S_AAP electrodes during the HER experiments. It can be observed that the catalytic activity of these electrodes is not as high as that of S_AGP .



Figure 4. Linear voltammograms recorded on the S_A , S_AG and S_AGP electrodes (a), as well as on the S_A , S_AA and S_AAP electrodes (b), in 1M KOH solution, at v = 5 mV/s

The electrochemical stability of the S_A , S_AG , S_AGP , S_AA and S_AAP electrodes during the H_2 evolution was also investigated. The first and 10^{th} voltammetry cycles obtained on these electrodes are shown in Figure 5 and evidence the fact that the most stable ones are S_AA and especially S_AGP . In their case, the minimum value of the current density reached during the HER when the first cycle is recorded doesn't suffer any significant changes by the time the 10^{th} cycle is obtained.



Figure 5. Cyclic voltammograms recorded on the S_A (a), S_AG (b), S_AGP (c), S_AA (d) and S_AAP (e) electrodes, in 1M KOH solution, at v = 5 mV/s

Conclusion

Using the experimental data obtained in strong alkaline medium during the OER and HER catalytic activity and electrochemical stability investigations of porous electrodes (modified with Ag, graphite, Ag and Pt, and graphite and Pt), it was possible to identify the most performant of them. In the case of the OER, the electrode consisting in a porous structure modified with graphite paste and Pt particles, inserted into the graphite support, is the most catalytically active. It also evidenced a high degree of stability during the electrochemical stability tests. The HER experiments aimed at finding the most catalytically active porous electrode outlined the same type of porous structure - but inserted into the Ag support. Furthermore, the electrochemical stability tests revealed this electrode to be the most stable. However, there are some issues that need to be addressed regarding the modified porous material in question and they will be the subject of future studies.

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ELECTROCHEMICAL DURABILITY OF MAGNETITE AND BIRNESSITE MODIFIED ELECTRODES WITH POTENTIAL APPLICATION IN WATER SPLITTING

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Abstract

Graphite electrodes were modified with compositions containing either Fe_3O_4 or δ -MnO₂, and their electrochemical durability was investigated using the cyclic voltammetry method. Experimental results indicate that the most stable electrode is the one modified with the composition containing magnetite and Vulcan carbon, when exposed to electrochemical potentials in the anodic domain. Given this result and the potential values at which oxygen is evolved on the electrode, it has the prospect to find application in the water splitting domain.

Introduction

With the increase in energy demand and with a global concern for the environment, the search for sustainable and renewable energy sources aimed at replacing fossil fuels has become especially important. A range of *energy* sources, including renewable ones, can be employed to generate hydrogen, which in turn is used for energy storage purposes. One way to generate hydrogen is *via* electrochemical water splitting. The two half-cell reactions taking place during this process – the oxygen evolution reaction (OER) and the hydrogen evolution reaction (HER) – require a practical amount of energy in order to unfold that can be decreased with the help of catalysts. In the past five years low-cost materials with high electrocatalytic activity for the OER and HER were discovered that have the potential to replace the standard noble metal-based catalysts [1-5].

The results presented in this paper are part of a larger study aimed at developing modified electrodes using materials with electrocatalytic properties for the OER and HER. A high electrochemical durability is a characteristic that electrocatalytic materials are currently expected to possess. In this context, three graphite electrodes were modified with compositions containing either Fe₃O₄ or δ -MnO₂ and their durability was evaluated by recording sets of cyclic voltammograms. The most stable electrode is the one modified with the composition containing Fe₃O₄ and Vulcan carbon, and it has the potential to be used for electrochemical water splitting.

Experimental

Magnetite and birnessite were synthesized hydrothermally. Carbon Black – Vulcan XC 72 (Fuel Cell Store), Nafion® 117 solution (Sigma Aldrich) and KOH (analytical grade, Merck) were also used in the study. The conductive substrate modified to obtain the electrodes was spectroscopic graphite type SW.114 ("Kablo Bratislava", Slovakia). All solutions were obtained using double distilled water. The modified electrodes were manufactured by drop-casting on the surface of graphite substrates volumes of 10 μ L collected from suspensions in ethanol having different compositions. The three electrodes evaluated in terms of their electrochemical durability were selected from a series of magnetite-based electrodes and a series of birnessite-based electrodes after preliminary experiments regarding their OER and HER catalytic activity.

The compositions used to obtain the modified electrodes are presented in Table 1.

Electrode label	Compositions used to obtain the electrodes						
	Fe ₃ O ₄	δ-MnO ₂	Vulcan carbon	Nafion solution			
	[mg]	[mg]	[mg]	[µL]			
G1	2	-	2	-			
G2	-	4	-	10			
G3	-	2	1	10			

Table 1. Modified electrodes labels and the compositions applied on the graphite substrates

Cyclic voltammetry experiments were performed using a glass cell equipped with three electrodes connected to a potentiostat. The reference electrode was the Ag/AgCl (sat. KCl) electrode and the auxiliary electrode was a Pt plate ($S_{geom} = 0.8 \text{ cm}^2$). Each modified electrode was used as working electrode ($S_{geom} = 0.28 \text{ cm}^2$). Electrochemical potential (E) values were expressed in terms of the Reversible Hydrogen Electrode (RHE) and the conversion was performed using the equation employed by Zhao *et al.* [6]. All experiments presented in this paper were performed in 1M KOH solution.

Results and discussion

The cyclic voltammograms recorded on the modified electrodes are shown in Figure 1. Since the G1 electrode displays catalytic activity for both OER and HER, its electrochemical durability was investigated in both the anodic and cathodic domain. In the former domain (Figure 1a), the durability of the G1 electrode was studied by recording 150 voltammetry cycles in a potential range in which the 10 mA/cm² current density (i) value is reached. In OER experiments this is the value at which the OER overpotential is usually specified [7]. When the first cycle is recorded, the E value corresponding to i = 10 mA/cm² (on the anodic branch) is ~ 1.74 V. This value is higher at the 5th cycle and lower at the 100th cycle. When the 150th cycle is obtained the E value becomes ~ 1.72 V. Thus, by recording 150 voltammograms the electrochemical potential corresponding to the specified i value decreases, indicating an improvement in the OER catalytic activity of the electrode. With regard to the maximum i value attained during O₂ evolution, a slight increase is observed from ~ 21 mA/cm² (the first cycle) to ~ 23 mA/cm² (the 150th cycle). The absence of significant change in the maximum i value indicates the relatively high electrochemical stability of the electrode.

The electrochemical durability of the G1 electrode was investigated in the cathodic domain as well (Figure 1b), by recording 120 voltammetry cycles. The shift in the potential value corresponding to the -10 mA/cm² current density was monitored throughout the experiment, and the following observations were made: In the case of the first cycle, on the cathodic branch of the voltammogram, the E value corresponding to i = -10 mA/cm² is about -0.43V. As the number of recorded voltammograms increases this E value gradually decreases, until it becomes ~ -0.52 V (120th cycle). The minimum i value obtained for the first cycle during H₂ evolution is ~ -18 mA/cm². At the 5th cycle the value becomes -20.2 mA/cm², then it increases to -11.5 mA/cm² (the 100th cycle) and subsequently to -10.5 mA/cm² (the 120th cycle). These results indicate that after the tracing of the 120 voltammograms the HER catalytic activity of the electrode depreciates significantly and its electrochemical stability in the investigated cathodic range is poor.



Figure 1. Cyclic voltammograms (iR corrected) obtained in 1M KOH solution, at a scan rate of 5 mV/s, on the (a) G1 electrode, in the anodic domain; (b) G1 electrode, in the cathodic domain; (c) G2 electrode, in the cathodic domain and (d) G3 electrode, in the anodic domain

Regarding the G2 electrode (Figure 1c), because it displays catalytic activity for the HER its durability was studied in the cathodic domain by recording 100 cycles. The E value corresponding to $i = -10 \text{ mA/cm}^2$, observed on the cathodic branch of the first cycle, is about -0.43 V. This value decreases (as can be seen on the 5th cycle) and then it increases up to -0.39 V (at the 100th cycle), indicating that the recording of the voltammetry curves improves the HER catalytic activity of the electrode. The minimum value of the current density obtained for the first cycle during H₂ evolution is -14.2 mA/cm². This value suffers a slight increase (the 5th cycle), but by the 100th cycle it becomes -22 mA/cm². Even though it should remain about the same throughout the voltammetric study in order for the electrode to be electrochemically stable, it is worth noting that the providing of a low current density value is desirable in HER studies [8].

Since it displays OER catalytic activity the electrochemical durability of the G3 electrode was evaluated in the anodic domain (Figure 1d). A total of 450 voltammetry cycles were obtained on this electrode, and the data show that the potential value corresponding to the 10 mA/cm² current density keeps increasing, starting from ~ 1.76 V and stopping at 1.785 V. This indicates that the OER catalytic performance of the electrode decreases. The maximum current density value obtained during the O₂ evolution, in the case of the first cycle, is ~ 29 mA/cm². This value keeps decreasing as the remaining voltammograms are traced and it becomes 25.6 mA/cm² (5th cycle), then 18.5 mA/cm² (100th cycle), 17.5 mA/cm² (150th cycle), and eventually 16 mA/cm² (450th cycle). Basically, the electrode has poor electrochemical stability in the given experimental conditions.

Conclusion

The electrochemical durability study performed on the G1, G2 and G3 modified graphite electrodes has led to the identification of the most stable electrode. This is the electrode obtained by drop-casting on the surface of the graphite substrate a composition containing both magnetite and Vulcan carbon, denoted G1. However, even though it displays a relatively good catalytic

activity for the OER and HER, its electrochemical stability is restricted to the anodic domain. Because of this, the electrode has the potential to be used as OER catalyst in the water splitting field.

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MORPHOLOGICAL INVESTIGATION OF CALCIUM PHOSPHATES ELECTRODEPOSITED ON TI IN THE PRESENCE OF TARTARIC ACID

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Abstract

The electrochemical deposition method was employed to deposit calcium phosphates on Ti substrates in the presence of different concentrations of tartaric acid. Scanning electron microscopy was used to investigate the morphology of the obtained specimens. The micrographs indicate that as the tartaric acid concentration increases, the size and number of calcium phosphate irregular structures increases as well. At the highest concentration a new morphology is evidenced, represented by flat ordered structures with high aspect ratio.

Introduction

Calcium phosphates constitute a class of ceramic materials with applications in domains such as orthopedics, dentistry, catalysis, in the manufacturing of fuel cells and gas sensors, in corrosion protection and the development of drug delivery systems [1-4]. The most studied and application wise useful member of this class is hydroxyapatite (HA) - $Ca_{10}(PO_4)_6(OH)_2$ - a biocompatible material with composition similar to that found in human bones and teeth [5]. The present work is a continuation of a previous study by Bucur *et al.* [6] in which HA was synthesized in the presence of tartaric acid *via* the hydrothermal method. The use of organic acids in the synthesis of calcium phosphates affects the morphology and properties of the resulted structures. Specifically, depending on concentration, they impoverish or prevent crystal growth on at least one crystallographic direction, by forming a complex with the Ca ion [7,8]. In this study, we replaced the hydrothermal method with electrochemical deposition. The latter is known to be an alternative way for obtaining calcium phosphates in general and HA in particular [9].

Experimental

Analytical grade $Ca(NO_3)_2 \cdot 4H_2O$ (Sigma Aldrich) and $(NH_4)_2HPO_4$ (Merck) were used as Ca and P precursors, in the presence of tartaric acid (racemic, Merck). The Ca:P molar ratio between the solutions was 1.67, corresponding to stoichiometric HA. Double distilled water was used throughout the study. Polished and cleaned Ti discs, cut from pure Ti plate, were employed as substrate for calcium phosphates electrodeposition. The electrochemical setup for obtaining the specimens consisted in a VoltaLab PGZ 402 potentiostat, three electrodes and a glass cell with heating mantle connected to a thermostat set at 80 °C. Each Ti disk was inserted into a support and used as working electrode ($S_{geom} = 0.28 \text{ cm}^2$). Every electrodeposition experiment lasted for 1h and was performed at a constant potential of -1.5 V. The electrolyte solutions used to obtain the specimens consisted in a mixture of the two precursors and tartaric acid, having the concentrations and volumes presented in Table 1.

Scanning electron microscopy (SEM) micrographs of the specimens were acquired with a Phillips Inspect S scanning electron microscope.

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	Electrolyte solution							
Specimen	Ca precursor		P pre	ecursor	Tartaric acid			
code	Volume	Conc.	Volume	Conc.	Volume	Conc.		
	[mL]	[mM]	[mL]	[mM]	[mL]	[mM]		
S 1	50	0.875	50	0.525	2.5	0.875		
S2	50	0.875	50	0.525	5	0.875		
S 3	50	0.875	50	0.525	10	0.875		
S4	50	0.875	50	0.525	15	0.875		
S5	50	1.75	50	1.05	2.5	1.75		
S6	50	1.75	50	1.05	5	1.75		
S7	50	1.75	50	1.05	10	1.75		
S8	50	1.75	50	1.05	15	1.75		

Table 1. S	necimen	codes a	nd the	electrolyte	solutions	used to	obtain them
10010 1.0	peemien	coucs u	ina uno	cicculoryte	solutions	useu to	ootum mem

Results and discussion

The morphological investigation *via* SEM imagery of specimens S1 to S4 indicates the following: In the case of S1, the Ti surface is covered with disconnected and sparse irregular structures having micrometric dimensions. By doubling the tartaric acid quantity in the electrolyte solution, the main observed change is an increase in the size of the micrometric irregular structures. As the acid solution amount is further increased, the surface of the Ti substrate becomes covered to a higher degree with calcium phosphate irregular structures that are bigger in size. In the case of S4 these structures are revealed to be porous and complex, but they do not organize into higher order symmetrical formations.

The SEM micrographs recorded for specimens S5 - S8, obtained from electrolyte solutions containing concentrations of precursors and tartaric acid twice as high as those used for the S1 – S4 specimens, increase the understanding of how the organic acid affects the calcium phosphate structures morphology. As the tartaric acid concentration increases, so does the size of the electrodeposited irregular structures evidenced for the S5 – S7 specimens. The main difference is outlined in the case of the S8 specimen, where many flat and elongated ordered structures are observed (Figure 1a). It can also be seen that the surface of the Ti substrate is covered with a discontinuous layer of small irregular particles (Figure 1b).



Figure 1. SEM micrographs recorded on the S8 specimen (a and b)

The results acquired for the S8 specimen are in agreement with the previously mentioned literature data regarding crystal growth prevention by organic acids along at least one crystallographic direction. In the case of the observed ordered calcium phosphate structures, their growth occurred almost entirely in length and was significantly slowed down when it comes to their width and height.

Conclusion

Out of the eight electrolyte solutions used in the present study, only the one with the highest concentrations of precursors and tartaric acid leads to the formation of ordered structures. These structures bear the shape of elongated plates and their morphology is probably due to the influence of tartaric acid affecting crystal growth by complexing the calcium ion. Future studies will be aimed at identifying the calcium phosphates formed during the electrodeposition of the S8 specimen.

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OPTIMIZATION OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR POLYETHYLENE GLYCOL-COATED GOLD AND SILVER NANOPARTICLES

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Abstract

The use of polyethylene glycol modified nanoparticles is becoming an interesting topic since they present a very good stability in biological media. However, the effects of these nanoparticles on organisms are still unclear, so it is necessary to monitor their presence in bodily fluids, such as plasma or urine. Single-particle ICP-MS is a versatile tool to simultaneously detect and characterize nanoparticles in aqueous media, but a previous extraction step is necessary when analyzing complex samples due to the occurrence of matrix effects. In this work, an ultrasound assisted dispersive liquid-liquid microextraction method based on the use of chloroform as extracting solvent has been optimized for the extraction, characterization, and quantification of polyethylene glycol modified gold and silver nanoparticles in aqueous media. So far, we could achieve extraction efficiencies higher than 75% for both types of nanoparticles studied. We believe that with further optimization, the extraction can be made quantitative.

Introduction

In recent years, the synthesis of surface modified nanoparticles (NPs) has become a hot topic. [1-3] One of the most recent and interesting ones are the polyethylene glycol-modified gold and silver nanoparticles (PEG-AuNPs and PEG-AgNPs, respectively), since they are very stable in biological media and can be used in numerous ways [4]. Although these PEG-modified NPs have a good potential in medical, biochemical, and biological applications, their effects on organisms are still unclear [5]. For this reason, it is necessary to monitor these NPs in bodily fluids during application.

Single particle inductively coupled plasma mass spectrometry (spICP-MS) is one of the most flexible techniques for NPs determination, since it allows the simultaneous quantification (i.e., mass and number concentration) and characterization (i.e., size distribution, structure, and composition) of a large variety of NPs. However, these analyses can be affected by matrix effects when introducing complex samples [6].

A classical strategy used to overcome these matrix effects is liquid-liquid extraction. Up to date, and regarding microextractions of NPs, only the cloud-point extraction (CPE) has been studied and optimized [7]. Although CPE can provide quantitative recoveries, this approach is time-consuming, and the heating step can negatively affect the NPs structure and surface molecules.

As an alternative, dispersive liquid-liquid microextraction (DLLME) can be suggested to be used, as it is faster than CPE and requires no heating or aggressive steps that can compromise the integrity of NPs. However, no studies have been published yet with regards to this. The objective of this work was to develop and optimize DLLME for the extraction of PEG-AuNPs or PEG-AgNPs from aqueous samples and quantify and characterize the NPs present in the extracts by means of spICP-MS.

Experimental

Reagents and solutions

All solutions and suspensions were prepared in ultrapure water. A stock suspension of monodisperse PEG-modified AgNPs (nominal diameter 50 nm) was obtained from NanoComposix (San Diego, USA). Chloroform and methanol were obtained from VWR Chemicals (Radnos, USA). Trisodium citrate sesquihydrate was purchased from Alfa Aesar (Ward Hill, USA), and chloroauric acid and thiol-modified polyethylene glycol were obtained from Sigma-Aldrich (Budapest, Hungary). Gold-containing precious metals solution ($10 \ \mu g \cdot mL^{-1}$) and silver-containing multielemental solution ($10 \ \mu g \cdot mL^{-1}$) were purchased from Inorganic Ventures (Spetec, Germany).

Synthesis and characterization of PEG-AuNPs

Polyethylene glycol modified AuNPs were synthesized according to the procedure described in [8], used here with slight modifications. Briefly, 735 μ L of 250 μ M chloroauric acid was added into a flask, mixed with 97 mL of ultrapure water, and heated up to 80°C. Next, 2 mL of 230 mM trisodium citrate was dropped to the mixture under mild stirring to reduce gold and produce citrate-capped AuNPs. After refluxing for 15 min, the suspension was slowly cooled to room temperature. For the surface modification of these AuNPs, 6 mL of the NP suspension was mixed with 6 mL of a 1.5 μ M thiol-modified PEG suspension under mild stirring. After 3h of reaction, the resulting suspension was washed to remove any unreacted reagents and was then kept at 4°C until use. PEG-AuNPs were characterized with an Ocean Optics Chem 2000-UV-Vis diode array absorption spectrometer, with a Philips CM-10 transmission electron microscopy (TEM) operating at 100 kV acceleration voltage, and via the dynamic light scattering method (DLS) on a Zetasizer Nano ZS Zen 4003 (Malvern Instrument, UK).

ICP-MS instrumentation and data evaluation

All spICP-MS measurements were performed employing a 7700x ICP-MS (Agilent, USA) instrument with the conventional sample introduction system. Isotopes monitored were ¹⁹⁷Au⁺ and ¹⁰⁷Ag⁺. The data acquisition was done in time-resolved analysis (TRA) mode. The measurement time was set to 120 s, with a dwell time (i.e., integration time) of 6 ms. To avoid occurrence of events associated to two or more nanoparticles reaching the detector at the same time, a sample flow rate of 600 μ L·min⁻¹ was chosen for all measurements. Transport efficiency was determined daily with the aid of the PEG-AgNPs via the particle frequency method [9] and found to be $\approx 2.5\%$ for all nanoparticles under study. Microsoft Office Excel software was employed to integrate event signals manually. Separation of events from background signals was carried out by manually selecting background threshold. The number of particle events found can be related to the NP concentration using the following expression [10]:

$$C_{NP} = \frac{n_{NP} \cdot 60}{\eta_n \cdot Q_l \cdot t_{Scan}}$$

where C_{NP} = particle number concentration (mL⁻¹); n_{NP} = number of particles detected; η_n = nebulization efficiency; Q_l = sample uptake rate (mL min⁻¹); t_{Scan} = measuring time (s⁻¹). The intensity of each individual event can be related to a nanoparticle diameter using the expression:

$$D_{NP} = 10^4 \cdot \sqrt[3]{\frac{6 \cdot I_{NP} \cdot t_{Dwell} \cdot Q_l \cdot \eta_n \cdot f_a}{\pi \cdot \rho_{NP} \cdot b_{Cal} \cdot 60}}$$

where I_{NP} = neat particle signal intensity (counts); t_{Dwell} = Dwell time (s); f_a = mass fraction of analyte in the NP; ρ_{NP} = nanoparticle density (g mL⁻¹); b_{Cal} = ICP-MS signal for a solution standard (counts L µg⁻¹).

Dispersive liquid-liquid microextraction

In this procedure, 4 mL of ultrapure water containing $7.0 \cdot 10^4$ mL⁻¹ PEG-AgNPs or $2.5 \cdot 10^4$ mL⁻¹ PEG-AuNPs were placed in glass test tubes. Two different extracting solvents have been tested, namely: *i*) chloroform; and *ii*) *n*-hexane. These two solvents have been selected based on their different polarity and similar volatility as well as good mixing with methanol. For the extracting solvent's volume we tested four different volumes: *i*) 250 µL; *ii*) 500 µL; *iii*) 750 µL; and *iv*) 1000 µL. In all cases, the extracting solvent was mixed with 1000 µL methanol and injected into the sample. Following this the test tube was immersed into an ultrasonic bath for 5 - 10 min. A cloudy dispersion was formed and, after 30 s of vortex stirring, the extractant droplet aggregated and was transferred into another glass test tube for complete solvent and extractant evaporation at room temperature in a fume hood. The solid residue was then resuspended in 4 mL of ultrapure water with the aid of an ultrasonic bath and directly introduced into the ICP-MS for NPs quantification and characterization.

Results and discussion

Characterization of the synthesized PEG-AuNPs

For a first observation of the PEG-AuNPs, UV-Vis absorption spectroscopy measurements were performed. The absorbance maximum of the plasmon band was found at 520 nm for both the citrate-capped AuNPs and the PEG-AuNPs, indicating that no aggregation or NP degradation had occurred. The core shape and size of the PEG-AuNPs was checked by TEM. All NPs presented a spherical size - ideal for spICP-MS characterization - and a core diameter of 26 nm with a standard deviation of 6 nm. Additionally, a DLS characterization was also carried out. The results of this characterization showed that the hydrodynamic diameter of the PEG-AuNPs was 42 nm, while for the citrate-capped AuNPs it was 30 nm, thus the AuNPs were successfully pegylated.

Optimization of the extraction parameters

First of all, it was necessary to demonstrate that we could still detect and characterize the pegylated NPs after the extraction, evaporation, and reconstitution steps. To this end, a suspension containing PEG-AgNPs in a concentration of 80,000 mL⁻¹ was extracted with 250 μ L of chloroform, as described in the corresponding section of the experimental procedure. For achieving the best extraction efficiency for PEG-AgNPs and PEG-AuNPs, the main strategy consists of making the NPs to have more affinity for the extracting solvent that for the aqueous media. Since the polyethylene glycol molecules are very stable in a wide range of pH and ionic strength values (i.e., salt concentration), the main parameters affecting the extraction of these nanoparticles are: *i*) type of extracting solvent, since it should have good affinity to polyethylene glycol; *ii*) extracting solvent volume, as it has to be enough to extract the NPs present in the suspension; and *iii*) the use or absence of sonication since it may help in dispersing the extracting agent thereby increasing the specific surface area of the droplets. In order to optimize these parameters, a series of experiments were carried out. Figure 1 shows the NPs

recovery for PEG-AuNPs and PEG-AgNPs by using different volumes of chloroform and hexane.



Figure 1. Recovery values for the extraction of (**■**) PEG-AgNPs and (**■**) PEG-AuNPs by using different volumes of (A) chloroform and (B) hexane, without sonication. Error bars represent standard deviations based on three replicate measurements.

As can be observed in Figure 1, chloroform provides a NPs extraction recovery of up to 25%, with a maximum efficiency when using 750 μ L of extractant. It is interesting to remark that there is a slight decrease of the recovery when using 1000 μ L of chloroform, probably due to the difficulty in dispersing such volume of dispersing solvent. With hexane, extraction efficiencies are far from chloroform values, with a maximum of 2.5%. This can be explained by considering that hexane is a purely apolar solvent and PEG is a hydrophilic polymer. In all cases, the mean diameter calculated for all detected nanoparticles is the same that the one obtained for the suspension before the DLLME step, so no dissolution or aggregation occurred during the extraction process. The extraction efficiences were generally quite similar for Au and Ag NPs, in accordance with the fact that their coating should determine their affinity towards the extraction solvent. However, in two hexane cases, the recovery was interestingly significantly different for Au and Ag NPs. It has to be added though that hexane recoveries were so low that these differences in recovery values can be negligible (e.g. ca. 1% as opposed to 2%).



Figure 2. Recovery values for the extraction of (■) PEG-AgNPs and (■) PEG-AuNPs by using sonication. Error bars represent standard deviations based on three replicate measurements.

In order to improve the extraction efficiency of the DLLME using 750 μ L of chloroform as extracting solvent, a sonication step was performed after the extractant injection into the sample. The effect of three short sonication times were evaluated: *i*) 0 min, *ii*) 5 min and *iii*) 10

min. Figure 2. shows the recoveries obtained for PEG-AgNPs and PEG-AuNPs operating this way.

As can be observed, NP extraction recoveries rose to 75% for both NPs under study when sonication with 10 min duration was employed, thus indicating that ultrasound helps in forming the chloroform suspension. It is important to remark that no NPs aggregation or dissolution was found as a consequence of the sonication step. However, further efforts need to be made in order to improve extraction efficiencies up to quantitative values (i.e., higher than 85% recovery). For this purpose, higher sonication times (e.g., 15 min) or frequency, or alternative extracting solvents with more polarity than chloroform (e.g., dichloromethane, tetrahydrofuran-decanoic acid micelles, ionic liquids, etc.) could be tested.

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EFFICIENT SYNTHESIS AND DETAILED THERMAL STUDIES OF ZINC PHTHALOCYANINE

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Abstract

A facile and efficient, one step synthesis of macrocyclic molecule zinc phthalocyanine (ZnPc), was proposed. Dark violet crystals of ZnPc dye were obtained in high boiling point solvent of dimethylformamide (DMF) in the presence of 2,2,6,6-tetramethylpiperidine (TMP) as a catalyst. Detailed thermogravimetric and kinetic analysis revealed high thermal stability of the investigated compound.

Introduction

In today's world, it's hard to find scientist who hasn't worked or be in touch with organic dyes. As a result, complex structures of metallophthalocyanines (MPs) with near-infrared incidence continue to pique the study community's curiosity. Phthalocyanines (Pcs) are aromatic heterocycles consisting of a planar conjugated system with 18 π -electrons and 4 isoindole subunits bridged by meso positioned nitrogen atoms. They feature a distinctive UV-Vis absorption spectrum with two primary bands, the weak Soret band at 300-400 nm and the Q-band located at around 600-800 nm. A compelling MPcs chemistry experienced its greatest growth in last two decades, revealing molecules that meet the high demands of photodynamic therapy (PDT), chemical sensor technology, non-linear optics (NLO) and dye-sensitized solar cells (DSSC). Furthermore, they have been used as electrocatalysts in fuel-cell reactors for dioxygen reduction. Notably, the nature of the coordinated central metal ion has a big impact on their photochemical characteristics. Thus, singlet oxygen production is poor in MPcs with paramagnetic metal centers. Closed shell and diamagnetic ions, such as Zn^{2+} , Ga^{3+} , and Si^{4+} , play a vital role in MPc complexes and contribute great features such as high singlet oxygen production, which is critical for photosensitizer PDT efficiency [1, 2].

The synthetic process for creating MPcs is constantly improving over the years based on the available research data [1, 3]. MPcs are frequently produced via cyclotetramerization of phthalonitrile or phthalic acid analogues, in the presence of a metal or metal salt, at high temperatures and reaction durations of several hours [2, 4]. Numerous investigations have been conducted to improve the synthesis approach of unsubstituted ZnPc, which is one of the most spectroscopically studied phthalocyanines [5]. On the contrary to the potential utility of these approaches, reaction condditions necessary for these reactions are very long reaction time and high temperature, resulting in a low obtained macromolecule yield.

In order to obtain a new and efficient catalysts for the ZnPc synthesis, adequate for organic solvents, we have examined the catalytic activity of 2,2,6,6-tetramethylpiperidine (TMP) in reaction of cyclotetramerization of phthalonitrile in dimethylformamide (DMF). Investigation toward extension of this procedure to other derivatives is in progress.

In addition, comprehensive thermal stability research of ZnPc crystals provides a realistic assessment for their real application.

Experimental

Dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and methanol were purchased from Merck. Zinc acetate dihydrate, 1,2-dicyanobenzene (phthalonitrile) and 2,2,6,6-tetramethylpiperidine (TMP) were purchased from Sigma Aldrich.

The ultraviolet–visible (UV/Vis) absorption spectra were recorded on a Shimadzu UV–Visible UV-2600 (Japan) spectrophotometer in the range 200–800 nm. FTIR spectroscopy measurements were performed on Nicolet TM 380 FT-IR spectrometer with Smart Orbit ATR accessory (Thermo Electron Corporation, Madison, U.S.A).

Thermogravimetric analysis of the ZnPc was tested on a TGA/DTA instrument (Setaram Setsys 1750 Evolution (France)). The sample was heated from 30 to 1000 °C in an atmosphere of pure argon (φ =20 cm³/min), with heating rates β = 5, 10 and 15°C/min. The average weight of the sample was 3 mg.

Synthesis

In a round bottom flask equipped with a reflux condenser and a magnetic stirrer, 2.5 mmol of phthalonitrile and 0.625 mmol of zinc acetate dihydrate were dissolved in 1 mL of DMF after which TMP was added. The reaction mixture was heated for 6 h at 153°C. After cooling to room temperature, the reaction mixture was precipitated by the addition of methanol. The precipitate was filtered off and washed with 2 mL of 3% HCl, water (20 mL) and methanol (15 mL). The resulting deep purple cristals remain after processing of reaction mixture.

Yield: 0.249 g (69%, $C_{32}H_{16}ZnN_8$). UV/Vis (DMSO): λ (nm) 670, 640, 603, 343. FT-IR, ν (cm⁻¹): 753, 775, 882, 1060, 1085, 1120, 1167, 1282, 1334, 1445.

Results and discussion

The synthesis of ZnPc was realized by a one step procedure starting from 1,2-dicyanobenzene in the presence of zinc acetate dihydrate and DMF as a solvent (Scheme 1.). After 6 hours of heating, deep purple ZnPc crystals were obtained in a high yield of 69% in the presence of an organic base, TMP, which acted as an excellent catalyst for the cyclotetramerization process.



Scheme 1. General synthetic scheme for the synthesis of zinc phthalocyanine (ZnPc) with precursors (a) Zn(CH₃COO)₂·2H₂O, TMP, DMF

The resulting purple crystals of metallophthalocyanine were characterised using the spectroscopic methods including UV-Vis and FT-IR. The UV-Vis absorption spectrum of macrocyclic compound ZnPc was shown in Figure 1. As can be seen from the spectrum there are two groups of absorption bands at 550–700 nm and 300–450 nm, respectively. The band from 550 to 700 nm were characteristic Q-band absorption of ZnPc. In this band group, the two well resolved absorption peaks at 670 and 603 nm should be assigned to the π - π * transition of monomer from the HOMO to the LUMO of the Pc⁻² ring [6]. Notably, between the two peaks

of Q bands, there appeared a weak peak at 640 nm, which might be assigned to the vibronic band due to the dimers and multimers while the band of 300–450 nm should belong to the typical B-band absorption of ZnPc arising from the deeper π levels-LUMO transition [7].



Figure 1. UV-Vis absorption spectra of ZnPc in DMSO at room temperature

The FT-IR spectral measurement is carried out to ascertain the functional group structures for ZnPc. The absorption peaks at 1334 cm⁻¹, 1167 cm⁻¹, 1120 cm⁻¹, 1085 cm⁻¹, 882 cm⁻¹, 753 cm⁻¹ were assigned to Pc skeletal vibration. Absorption peaks observed around 1445 cm⁻¹ and 1282 cm⁻¹ were assigned to aromatic phenyl ring and C–N=strech vibrations. The peaks at 1060 cm⁻¹ and 775 cm⁻¹ are assigned the C–H in-plane bending vibrations and C–H out-of-plane bending vibrations, respectively. In the IR absorption spectra, the formation of Pc was confirmed by disappearance of phthalonitrile C=N band at 2226 cm⁻¹.

Thermal stability of ZnPc was analyzed using TGA-DTG and DTA techniques. The thermal stability of the sample is represented by the temperature at which its decomposition begins. TG curve (Figure 2a.) of compound shows that thermal degradation occurs in two steps. The first temperature loss at 50 °C is caused by evaporation of free adsorbed water which also can be seen on DTA curve (Figure 2b.) as an endothermic peak at around 50 °C.



Figure 2. TG (a) and DTA (b) curves for ZnPc at three different heating rates (5, 10 and 15 $^{\circ}C/min)$

The second temperature loss, between 500-700 °C, is due to the decomposition of Pc skeleton. These mass losses are observed as two peaks in DTG feature (Figure 3.) at 50 and 639 °C (for heating rate 5 °C/min) [8].



Figure 3. DTG curves for ZnPc at three different heating rates (5, 10 and 15 °C/min)

The kinetic parameters, pre-exponential factor (A) and apparent activation energy (E_a) for ZnPc molecular structure degradation process that occurred at the second steps are obtained by Kissinger (Eq. 1) [9] and Ozawa (Eq. 2) methods. These two methods are based on shift of the peak temperature T_{max}, with heating rate (β) changes:

$$\log \frac{\beta}{T_{max}^2} = \log \frac{AR}{E_a} - \frac{E_a}{2.303 \cdot RT_{max}}$$
(1)
$$\log \beta = \log \frac{AE_a}{R} - 2.315 - 0.4567 \left(\frac{E_a}{RT_{max}}\right)$$
(2)

A is determined from the intercept, while the E_a is determined from the slope of linear dependence log (β/T_{max}^2) –1/ T_{max} for Kissinger and log β –1/ T_{max} for Ozawa method. A high value of activation energy, over 700 kJ/mol, indicates exceptional thermal stability of the investigated compound. The value of the pre-exponential factor greater than 10^{16} min⁻¹ means that a large number of collisions of molecules as well as high values of E_a are required for the degradation of the compound.

Table 1 The value of A and	d Ea using Kissinger and	Ozawa method for ZnPc
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Sample	Kissinger method		Ozawa method		
	E _a , kJ/mol	A, min^{-1}	Ea, kJ/mol	A, min^{-1}	
ZnPc	730.67	4.00×10^{41}	709.20	6.85×10^{40}	

Conclusion

In conclusion, we have developed a convenient procedure for the high-yield synthesis of ZnPc employing TMP as an efficient catalyst. Refluxing phthalonitrile and zinc acetate in DMF and TMP, gave deep purple crystals of macrocyclic ZnPc compound. Noteworthily, this approach is currently being investigated to see whether it can be extended to other MPcs. The investigated compound's exceptional thermal stability was revealed by detailed thermogravimetric and kinetic analysis.

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CORROSION OF CARBON STEEL OLC 45 AND STAINLESS STEEL AISI 304L IN WINES FROM BANAT COUNTY

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Abstract

In the present paper, results on the corrosion rate of carbon steel OLC 45 (similar to EU C 45) and stainless steel AISI 304L in a few species of wines from Banat County (Romania) have been emphasized. Corrosion rates have been determined using weight loss measurements, as well as Tafel plots method. The effect of wines on the surface of studied steels has been revealed using scanning electron microscopy. The obtained results show that the values of the corrosion rates in the wines tested for OLC 45 are close to the maximum allowed limit, which means that this type of steel cannot be used in the wine processing and storage. On the contrary, the corrosion rates for AISI 304L steel are extremely low in all the analyzed wines, which proves that they can be used both for wine processing and storage.

Introduction

In the last years, Romania has recorded significant successes in wine production, both in terms of quantity and especially in quality. According to the estimate data, in 2020, the production volume was 3.6 million hectoliters, our country being the 13th rank in the world's largest wine producers [1]. The essential condition in the obtaining of a high quality wines is the preservation of the organoleptic features. Containers used during the alcoholic fermentation process or for wine storage are made of different materials such as stainless steel, plastics and wood. These materials significantly influence the composition of the end product, increasing the metals ion content. In some cases, the relatively high level concentration of heavy metals ions (Mn, Ni, Cu) requires their partial extraction using ion exchangers. These metals ions have their origin not only in the soils of the vineyards, but also from metallic materials of the equipment used during the vinification process and storage [2]. According to the present paper, in order to improve the quality of the wines and ensure a low content of metal cations, the corrosion rates of some steels used in different stages of the technological wine process have been determined in some varieties of wines from Banat County.

Experimental

Materials

For the wine samples used in the experiments pH, conductivity and sulfur dioxide content were previously determined (Table 1).

No.	Wine type	Symbol	рН	Conductivity [S m ⁻¹]	SO ₂ content [mg L ⁻¹]
1	Muscat Otonel 2016 half-sweet	W1	3.34	174 x 10 ⁻³	160
2	Fetească Regală 2016 half-sweet	W2	3.24	194 x 10 ⁻³	175
3	Sauvignon Blanc 2016 dry	W3	3.14	193 x 10 ⁻³	180
4	Rose 2016 half-dry	R1	3.01	157 x 10 ⁻³	145
5	Cabernet Sauvignon 2016 dry	R2	3.29	192 x 10 ⁻³	125

Table 1. Characteristics of the wines used in the experiments.

In the experimental tests, two types of metallic samples were used: carbon steel OLC 45 and food grade stainless steel AISI 304L. Their elemental composition is given in the table 2 and table 3.

 Table 2. Elemental composition of the carbon steel OLC 45.

Element	Fe	С	Si	Mn	Р	S	Cr	Ni
wt %	96.98	0.4184	0.2510	0.7920	0.0132	0.0335	1.162	0.029
Element	Мо	Cu	Al	Ti	V	Со	Nb	W
wt %	0.2123	0.0234	0.0229	< 0.004	0.0124	0.0222	< 0.001	< 0.010

Table 3. Elemental composition of AISI 304L stainless steel.

Element	Fe	С	Si	Mn	Р	S	Cr	Ni
wt%	64.89	0.030	1.000	2.00	0.05	0.030	20.0	12.0

Corrosion rate evaluation by weight loss measurement

This method consists in the complete immersion of the metal sample in the corrosive environment and determination of the weight loss after 21 days using an analytical balance. The amount of corroded metal is obtained according to the relationship (1).

$$K = \frac{m_i - m_f}{S \cdot t} \tag{1}$$

in which K is the corrosion rate, in g m⁻² h⁻¹; m_i – initial mass of the metal sample, in g; m_f – final mass, in g; S – sample area, in m²; t – test time, in h.

The accuracy of the method is conditioned by the possibility of complete removal of corrosion products from the sample surface.

Corrosion rate evaluation by Tafel plots method

The corrosion rate can also be expressed by the corrosion current density i_{cor} . This amount is determined by the Tafel slope method, based by drawing the metal sample potential in the corrosive environment as a function of the current density flowing through the interface [3,4]. By knowing the value of the corrosion current density, one can calculate the corrosion rate *K*, expressed in g m-2 h⁻¹, based on the relationship (2).

$$K = \frac{A}{zF} \cdot i_{cor} \cdot 3600 \tag{2}$$

in which A is the atomic mass of the basic metal; z - metal ion charge; F – Faraday's constant, in C mol⁻¹; i_{cor} - corrosion current density, A m⁻².

If the corrosion process is homogeneous, the corrosion rate can also be expressed by the penetration rate, expressed in mm year⁻¹, according to relationship (3).

$$K_p = \frac{24 \cdot 365 \cdot K}{\rho} \tag{3}$$

in which ρ is the metal density, in g mm⁻³.

The volume of the test solution has to be large enough so that changes of the concentration are minimal during the tests.

Scanning electron microscopy was used to study the morphology of the surfaces subjected to the action of wines. An SEM Quanta 250 FEG was used.

Results and discussion

The corrosion rates of OLC 45 and AISI 304L steels, determined by the weight loss measurements after 21 days are shown in Table 4.

No.	Wine type	Corrosion rate $[g m^{-2} h^{-1}]$						
		OLC 45	AISI 304 L					
1	W1	0.0368	0.0002					
2	W2	0.0314	0.0000					
3	W3	0.0430	0.0002					
4	R1	0.0974	0.0004					
5	R2	0.0531	0.0000					

Table 4. Corrosion rates determined by weight loss measurement.

It can be seen that OLC 45 carbon steel is much less corrosion resistant in the studied wines than AISI 304L stainless steel. For the last steel, in wines W2 and R2, the weight loss mass was below the detection limit.

Furthermore, the corrosion current densities were determined by plotting the Tafel polarization diagrams, shown in figures 1 - for the OLC 45 carbon steel and in figure 2 - for AISI 304L steel. The electrochemical parameters and the corrosion rate calculated based on the Tafel plots are shown in tables 5 and 6.



Figure 1. Tafel polarization plots drawn on OLC 45 carbon steel in different wine types, at a temperature of 25°C and scan rate of 1 mV s⁻¹.

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Tuble 5. Electrochemical parameters acterimited by micar polarization on OEC 45.								
Wine type	T [K]	$i_{\rm cor}$ [µA cm ⁻²]	E _{cor} [mV]	$-b_{\rm c}$ [mV dec ⁻ ¹]	b_a [mV dec ⁻ ¹]	$R_{ m p}$ [Ω]	v_{cor} [mm an ⁻ ¹]	
W1		35.3	-526	131	231	1260	0.414	
W2		37.5	-528	129	234	1170	0.439	
W3	298	35.0	-522	122	223	1201	0.411	
R1		33.0	-521	128	235	1388	0.388	
R2		49.1	-588	167	252	942	0.576	

Table 5. Electrochemical parameters determined by linear polarization on OL	C	45
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Figure 2. Tafel polarization diagrams drawn on stainless steel AISI 304L in different types of wine, at a temperature of 25°C and at a scan rate of 1 mV s⁻¹.

Wine	Т	İcor	Ecor	- <i>b</i> c	ba	Rp	Vcor
type	[K]	[µA cm ⁻²]	[mV]	[mV dec ⁻¹]	[mV dec ⁻¹]	[kΩ]	[mm an ⁻¹]
W1		1.1	-322	222	108	25.3	0.013
W2		1.0	-304	179	102	29.5	0.011
W3	298	0.9	-291	172	105	31.6	0.010
R1		0.7	-254	134	113	34.2	0.009
R2		1.8	-362	284	107	17.1	0.021

Table 6. Electrochemical parameters determined by linear polarization on AISI 304L.

It is found that the corrosion currents for AISI 304L are about 30 times lower than for OLC 45. As well, the same ratio is recorded for polarization resistance, that is proportional to the corrosion resistance of the metal in the studied environments. Based on the values of the corrosion currents, the corrosion rates expressed in mm year⁻¹ were calculated. It is significant to emphasize the corrosive effects of wines on studied steels using the images obtained by scanning electron microscopy. Figures 3a and 3b show the SEM micrographs for OLC 45, respectively for AISI 304L.



Figure 3a. SEM micrography of OLC 45.

Figure 3b. SEM micrography of AISI 304L.

Additionally, SEM micrographs show that the surface of the carbon steel undergoes significant transformations in prolonged contact with the wine, while the surface of AISI 304L remains virtually unchanged.

Conclusion

Corrosion rates were determined by two methods for OLC 45 carbon steel and AISI 304L stainless steel. The corrosion rates obtained by the Tafel slope method are substantially higher as they represent quasi-instantaneous values from the beginning of the process, when the metal surface was not shielded by corrosion products. The obtained results show that the values of the corrosion rates in the wines tested for OLC 45 are close to the maximum allowed limit, which means that this type of steel cannot be used in the manufacturing of wine processing, storage devices and equipment. On the contrary, the corrosion rates for AISI 304L steel are extremely low in all the analyzed wines, which proves that they can be used both for processing and storing wines.

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DRIED FIGS AS SOURCES OF ESSENTIAL MICROELEMENTS

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Abstract

The paper aims to determine the concentration of some microelements essential in cardiovascular sistem health from dried figs and evaluate their mineral intake. The concentrations of Fe, Mn, Zn and Cu were determined from samples of dried figs from the local trade, imported from different countries in the Mediterranean areas.

The results obtained, by flame atomic absorption spectrometry (FAAS) method, show that these fruits contain important quantities of essential microelements, depending on fig providers: 22.1 - 32.5 mg/kg Fe, 4.14 - 12.73 mg/kg Mn, 4.53 - 10.20 mg/kg Zn and 3.95 - 9.16 mg/kg Cu.

The analytical results obtained allowed the evaluation of the mineral intake of these fruits for men and women aged between 19 - 50 years. Thus, a consumption of 40 g of dried figs covers a large part of the need for microelements, as follows: 13.99% Fe, 13.39% Mn, 2.83% Zn and 28.07% Cu - for men and 6.22% Fe, 17.11% Mn, 3.89% Zn and 28.07% Cu - for men.

These results show that the dried figs analyzed could be considered as sources with some essential microelements, especially in terms of Cu, Mn and Fe.

Introduction

Dried figs are a good source of carbohydrates, including fiber, sugars (mainly fructose and glucose), proteins, organic acids, phenolic compounds and phytochemicals, essential minerals, vitamins and enzymes.[1,2,4,6,8,10]. Previous studies indicate that dried figs are a good source of essential microelements: Zn, Fe, Mn, Cu, Se etc who play significant cardioprotective roles when they are present in adequate pharmacologic concentrations due to their antioxidant, antinflammatory and immune function modulatory [3,5].

Experimental

In this study we aimed to determine the concentration of Fe, Mn, Zn and Cu, microelements essential for the proper functioning of the cardiovascular system, from samples of dried figs sold in the local markets and evaluation of the mineral intake corresponding to a consumption of 40 g of figs. In order to determine the microelements, samples of dried figs (imported from countries in the Mediterranean area) were taken from five markets in Timisoara (Provider 1-5), from which the samples were made for the proper analysis. The Fe, Mn, Zn and Cu concentrations in the dried figs were determined by the Flame atomic absorption spectrometry method - the dried calcination variant and the solubilization of the inorganic residue in 0.5 M HNO₃ [11].

To determine the mineral intake, respectively the degree of coverage of the daily requirement with micronutrients, the daily requirement recommended by Fe, Mn, Zn and Cu, for people aged 19 - 50 years (table1) [3] and their concentrations (mean values) in 40 g were taken into account. uacate figs analyzed. Usually, 40 g (on a per serving basis) is a correct amount that can be considered as the average dried figs quantity consumed by an ordinary person [12].

Specification	Elements (mg/kg)						
specification	Fe Mn		Zn	Cu			
Provider 1	32.5±1.99	12.73±0.81	4.53±0.39	7.78±0.53			
Provider 2	29.2±1.87	4.14±0.49	9.75±0.56	3.95±0.37			
Provider 3	31.8±1.70	10.72±0.73	7.62±0.50	9.16±0.61			
Provider 4	24.3±0.98	5.24±0.48	6.81±0.47	5.47±0.40			
Provider 5	22.1±0.75	5.66±0.42	10.2±0.59	5.22±0.46			
Mean values	27.98	7.70	7.72	6.32			

Table 1 The concentration of Fe, Mn, Zn and Cu in dried figs from Mediterranean
countries

As shown in Table 1, microelement concentrations varies depending on fig providers. Fe is the best represented among the microelements, the average concentration having the value of 27.98 mg/kg. Lower but very close average concentrations were determined for Zn and Mn (7.2 and 7.70 mg/kg, respectively). Cu was identified in smaller amounts (6.32 mg/kg average value) than Fe, but close to Zn and Mn concentration.

Table 2. Mean mineral intake for men and women correspondingto a consumption of 40 g of dried figs

	Mineral intake [%]							
Specification		Me	en		Women			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
Mean value	13.99	13.39	2.83	28.07	6.22	17.11	3.89	28.07

According to Table 2, it can be stated that, from the point of view of insurance with essential microelements, the highest values were registered in the case of Cu (women and men), Mn (women and men) and Fe (women). Lower values were determined for Fe (women) and Zn (men and women).

Conclusion

The results of this research confirm that dried figs contain significant amounts of essential microelements that play significant cardio-protective roles.

The concentrations of the microelements in the analyzed figs show non-uniformity, depending on the origin and nature of the microelement.

The average concentrations of microelements in the analyzed dried figs show the following decreasing trend: Fe > Zn \cong Mn > Cu.

The results obtained when evaluating the mineral intake show that dried figs can be considered as sources of essential microelements, especially in terms of Cu, Mn and Fe.

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DEACTIVATION OF SOLID ACID CATALYSTS FOR ETHANOL DEHYDRATION REACTION

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Abstract

Heteropolycompounds (HPCs) has attracted much interest because of its potential to generate economic rewards and green benefits. Heteropolyacids (HPAs) are insoluble in non-polar solvents and therefore, can be used as green solid acids to replace environmentally unsafe traditional acid catalysts such as H_2SO_4 and HF [1,2].

In this study, 12-tungstophosphoric acid (HPW) and its cesium salts $Cs_xH_{3-x}PW_{12}O_{40}$ (x=1, 2, 2.25 and 2.5) were doped with palladium and supported on mesoporous silica molecular sieve SBA-15. The as-prepared catalysts were characterized by various techniques including thermogravimetric analysis (TG-DTA), FT-IR spectroscopy, X-ray diffraction, scanning electron microscopy (SEM) and BET.

The conversion of ethanol (Et-OH) into ethylene (ET) and diethyl ether (DEE) was investigated in a temperature range of 200-350 °C. The ET was the main product at high temperatures while DEE was formed at lower temperatures. The results show that higher catalytic activity, characterized by conversion and selectivity, can be achieve at optimal temperature in the range 275-300 °C.

One of the major problems is the loss of catalytic activity with time on stream due to deactivation processes at 300 °C. The formation of coke deposit cause poisoning and/or pore blockage of active sites [3]. The amount of coke precursor present in the catalysts was calculated by the difference between the initial mass of spent catalyst sample after isothermal heating at 300 °C (temperature of reaction test) and the sample mass heated in nitrogen at 650 °C. Soft coke is removed from the samples through volatilization in inert nitrogen and refers to high molecular weight aliphatic oligomers. The amount of hard coke present in the catalysts was calculated as the difference between samples mass heated in nitrogen at 650 °C and mass loss of sample at 650 °C in air, when the coke was burnt out. Hard coke refers to heavy polynuclear aromatics. The sum of coke precursor and hard coke represent the total coke.

The catalytic tests demonstrate that by adding the palladium there are no significant changes to the catalytic activity and coke formation of pure heteropoly compounds. By supporting the HPW and PdPW on mesoporous molecular sieve SBA-15 the catalytic activity in ethanol dehydration reaction was improved. Palladium doping of HPA/SBA-15 significantly decreases the formation of coke deposit. A fairly good correlation between coke formation and catalyst acidity has been established, which demonstrates that the Brönsted acid sites play an important role in HPCs deactivation.

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COMBINED EXPERIMENTAL AND DFT STUDY OF LITHIUM-INDIUM-OXIDE STRUCTURE AND VIBRATIONAL PROPERTIES

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Abstract

A promising lithium-indium-oxide (LiInO₂) wide band-gap semiconductor for scintillating detection, photoluminescence, and photocatalysis [1-3] was prepared by a mechanochemical solid-state synthetic procedure that can be found elsewhere [3]. Its structure and morphology were investigated by using X-ray diffraction (XRD), scanning electron microscopy (SEM), and Raman spectroscopy. SEM images show agglomerates of relatively uniform size of around 300 nm spherical-shaped particles of LiInO₂ powder, while the XRD pattern confirmed the formation of the nanocrystalline tetragonal structure with $I4_1/amd$ space group (no. 141) symmetry. Detailed vibration analysis, together with the assignments of the band modes, was performed through the best-fit match of the experimental and density functional theory (DFT) calculated Raman spectrum. Geometry optimizations and vibrational frequencies calculations were conducted using B97-1 functional correlation [4] and LanL2DZ was used as a basis set.

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WATER POLLUTION: COMPLIANCE ON GREASE TRAP USAGE AMONG FOOD PREMISES IN MALAYSIA

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Abstract

The study aims to explore motivation and barriers among restauranteurs to comply with new regulations to install a grease trap in their food premises in Malaysia. This study used an exploratory qualitative design. The 38 participants of the interviews survey were food premise's owner that had attended a discussion about grease trap usage on 28 September 2020 conducted by the Local Council of Baling. Verbatim transcripts were analyzed using thematic analysis based on Braun and Clark's six steps method. The results show awareness, benefits to individuals and society, and social influence as motivation factors. Meanwhile, technical factors, knowledge, financial, inconvenience, and enforcement emerged as barriers.

Introduction

Water protection is an essential element of environmental issues. Clean water and sanitation are highlighted as one of the Sustainable Development Goals of the United Nations. Availability and accessibility to clean water have to do with human civilization and human rights [1]. A relevant part of the world population still lacks access to clean water and sanitation in 2020 [2], while millions of people have lost their lives to water-borne related diseases such as diarrhea, cholera, dysentery, typhoid, and polio [3]. In Malaysia, the river is the primary source of water supply which contributes to 90% of the water supply [4]. Malaysia must face unprecedented challenges in the field. 53% of the rivers in Malaysia are polluted, and the trend is deteriorating [5].



Clean Slightly Poluted Poluted

Figure 1: River water quality in Malaysia between 2008 and 2017 [5]

According to a report conducted by the Department of Environment (DoE) under the Pollution Prevention and River Water Quality Improvement Program for the Eighth Malaysia Plan and the Ninth Malaysia Plan, it was found that food premises as one of the main contributors to river pollution [6]. As a result, proper grease management has become a significant waste management issue [7], and illegal disposal and improper management of fats, oils, and grease (FOG) discharge remain the primary source of river pollution cases in Malaysia [8]. Pollution occurs due to the discharge of wastewater with fat, oil, and grease content from the sink of food premises into the drainage system without any separation process [9]. For instance, used cooking oil from food premise sinks flowing into wastewater systems will cause problems to wastewater treatment plants or be integrated into the food chain through animal nutrition, thus becoming a potential cause of health problems to humans [10].

There are several approaches to environmental protection. Cleaner production [11] solutions aim to avoid water pollution, or the concept of circular economy [12] helps to reduce the amount of waste landfilled. We can acknowledge the benefits and long-term impacts of deeply agree that investments in these technologies, but it should be noted that the required efforts may be at limited availability. The investment needs may exceed the opportunities, or the lack of state aid also hinders the implementation of cleaner technology. In such cases, the less effective solution of the end-of-pipe protection is particularly appreciated. In a local society or a country where clean water gives the bottleneck of the urban system and well-being, filters can provide a leap forward before changing the technology.

Moreover, the results achieved may encourage further progress. The paper focuses on one device applicable in the study area. A grease trap (see Figure 2) is a device that serves to separate food waste, fat, oil, and grease from wastewater before the water is discharged to a sewer system or septic tank.



Figure 2. Cross-section of typical grease interceptor [13]

The installation of grease traps is one of the practical methods in dealing with the problem of pollution to the irrigation system [9]. Thus, the Guidelines for Grease Trap on food premises in local authority areas was prepared by the Local Government Department, Ministry of Housing and Local Government in 2005 and made it mandatory to use food waste and oil filters in food premises nationwide, it has become a part of the condition for the issuance and renewal of business licenses in local authorities [6].

Experimental

This paper explores the motivations and barriers faced by restauranteurs to install a grease trap in their food premises by asking them two specific key questions what motivates them to install a grease trap and the barriers to installing a grease trap. A total of 38 persons were purposively chosen for an interview. Participants were among those 61food premises that obtained grades B and C during the food premises grading assessment in 2019 due to not installing grease traps. Inclusion criteria for participation in the interview, participants, had attended a talk about grease trap conducted by the Local Council of Baling on 28 September 2020. Fieldwork observation, face to face and telephone interview was used as an instrument for data collection. Data from the interview were transcribed word by word. The participant's statement was openly coded through a thematic six-step analysis method [14].

Results and discussion

47.4% of the participants have installed a grease trap (marked as installers), while 52.6% still do not use one (marked as non-installers). Installers were asked further on what motivates them to install grease traps, and non-installers were asked about the hindrances they encountered while installing grease traps.

Main themes	Sub-themes	Number of responses	Percentage (%)
Awareness	Environmental cleanliness Prevent drain from clogging Awareness to keep the environment clean	12	50
The benefit to individual and society	Less complaint from society Easier to clean and manage	8	33.3
Social influence	Government Society	4	16.7

Table 1 Main themes and sub-themes for motivation among restauranteurs to install a grease trap in their food premises

Table 1 shows the thematic analysis revealed three main themes for motivation to install grease trap which composed of (i) Awareness: environmental cleanliness, prevent the drain from clogging and awareness to keep the environment clean; (ii) Benefit to individual and society; fewer complaints from society and easier to clean and manage; and (iii) Social influence: government and society. Based on the main themes count, awareness most frequently cited by participant (cited 12 times), followed by second reason labeled as a benefit to individual and society (cited 8 times), and third motivation is driven by social influence (cited 4 times).

Examples of the typical responses about motivation are as follows:

"To ensure oil, food waste not entering drain, I see others stall installed it as well so I installed also, I just want to ensure drain behind my stall not clogging due to oil and food waste."

"To keep the drain clean from oils and food waste and prevent clogging."

"If I do not install a grease trap, I had to clean drain every night and if I am not clean it will smell bad, and nearby stalls and people will complain as well."

"I received a complaint before from public due to bad smell from my stall drain so I decide to install grease trap to avoid that from happen again."

"Because it is promoted by the local council to install grease trap and many other stalls already install it."

Non-installers were asked what obstacles they encountered to install grease traps. The thematic analysis revealed five main themes, namely: (i) Technical: small space, type of building and use of the different type of filters, (ii) Knowledge: lack of knowledge and information, (iii) Financial: expensive cost of installation and business slow during MCO/pandemic, (iv) Inconvenience: challenging to clean and manage and (v) Enforcement: lack of enforcement.

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Main themes	Sub-themes	Number of responses	Percentage (%)
Technical	Small space Type of building Used different types of filters	7	30.4
Knowledge	Lack of knowledge and information	6	26.1
Financial	Expensive cost of installation Business slow during MCO/pandemic	4	17.4
Inconvenience	Difficult to clean and manage	4	17.4
Enforcement	Lack of enforcement	2	8.7

Table 2. Main themes and sub-themes for barriers to install grease traps among restauranteurs

The technical factor is the most common response given by non-installers (cited 7 times), which participant indicates that under their stall sink too small to fit in the grease trap, open space type of restaurant, and some of them used different types of food filters. The second typical response is a lack of knowledge and information regarding grease traps and how to install them. The third typical response is both financial and inconvenience. A small number of responses (cited 2 times) reported that they had not installed grease traps because of lack of enforcement, due to the local council made it mandatory to install a grease trap to apply for a business license for food premises. Examples of typical responses related to barriers encountered to install grease trap:

"My sink located at open space, I used to place grease trap before here but it is stolen since then I afraid to install it again."

"Space under my sink cannot fit the grease trap."

"In case I install it, it still no use because I did not know how to use it"

"I did not know which supplier I should contact to install grease trap, it would be easier if I knew who to contact to install it."

"This restaurant has been opened for 40 years already, since my grandfather generation and I am the third generation, we did not install grease trap and no problem so far."

"It cost me RM 800 includes the cost of the installation which is a lot for me since I could not open my business as usual due to MCO."

"My food business was badly affected by Covid, we could not manage to get revenue like we used to get before MCO."

"I opened a bakery stall, if I install grease trap flours will be accumulated and clumped together which make it difficult to clean later, so it easier for me not to install."

"I did not use much oil in this restaurant, we used to cook at home and bring food here to sell, we just used a simpler type of food filter on the sink."

Conclusion

The results suggest that installers are willing to install grease traps even though installation costs are quite expensive due to their awareness of environmental protection. On the other hand, the approach of non-installers will provide important insight for local authorities to take into consideration in order to improve participation among food premise owners in the future. It is expected that understanding people's motivation and obstacles will help overcome the waste management problems in Malaysia, and local authorities could provide an affordable solution and technical support to local restaurant owners as the first step towards sustainability.

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XANTHAN PRODUCTION ON GLYCEROL-BASED MEDIA: A MINI REVIEW

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Abstract

Xanthan is the most important microbiological polysaccharide. This biopolymer has widespread commercial applications as a viscosity enhancer, emulsifier and stabilizer in the food, pharmaceutical and petrochemical industries. The yield and properties of the produced xanthan are largely dependent on the producing microorganism, medium composition and process parameters. In industrial conditions, a pure bacterial culture is used to produce xanthan on a medium containing glucose or sucrose as a carbon source. However, the increasing market price and demand for these sugars indicate the need for implementation of an alternative raw material. Hence, many studies are focused on finding cheap and available, alternative raw materials, rich in carbon sources, as a replacement for mentioned sugars. Results from several studies indicate that certain strains of bacteria of the genus *Xanthomonas* possess the ability to biosynthesize xanthan on a medium with glycerol as the sole carbon source. The aim of this study is to discuss data from available scientific literature related to the xanthan production on glycerol-based media.

Introduction

Xanthan is non-toxic, biocompatible and biodegradable polysaccharide of microbial origin [1]. There are only a few microbial exopolysaccharides that are commercially available and xanthan is one of the most important [2]. Outstanding rheological properties of xanthan solutions contribute to its wide-range of applications as a suspending, stabilizing, and/or thickening agent in the food industry and its use as an emulsifier, thickening agent for oil recovery [3]. Xanthan is approved by the United States Food and Drug Administration (FDA) for use as a food additive without any specific quantity limitations and European Economic Community approved xanthan under the number E415 as a permitted thickener and stabilizer [4]. Currently leading xanthan manufacturers are Jungbunzlauer (Switzerland), ADM (USA), Cargill (USA), CP Kelco (USA), Deosen Biochemicals (China) and Meihua Group (China) [5]. The demands for xanthan are constantly growing and according to GlobeNewswire it is estimated that xanthan production will reach 1403 million US dollars by 2026.

This extracellular heteropolysaccharide has a primary structure consisting of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0 [6]. The structure of xanthan macromolecule is composed of linear chain consisting with 1,4-linked β -D-glucose backbone with two tri-saccharide units pairing the side chain on every glucose unit. The other, side chain is formed by a D-glucuronic acid unit-linked between two D-mannose units [7]. Structural characteristics directly affect xanthan molecular weight. The molecular weight of xanthan is usually from $2 \cdot 10^5$ - 10^6 Da [8].

Despite the fact that many different strains as Xanthomonas arboricola, Xanthomonas axonopodis, Xanthomonas campestris, Xanthomonas citri, Xanthomonas fragaria, Xanthomonas gummisudans, Xanthomonas juglandis, Xanthomonas phaseoli and Xanthomonas vasculorium have the ability to produce xanthan, X. campestris is the most

commonly strain employed for industrial production of xanthan [1]. On industrial scale, production of xanthan is usually performed by aerobic submerged batch cultivation of reference strain *X. campestris* ATCC 13951 on the appropriate medium under optimal conditions [9]. Glucose, sucrose, starch, sugarcane molasses and corn syrup are most commonly used as carbon sources in biotechnological production of xanthan, but the industrial production of xanthan is mainly based on the usage of glucose or sucrose containing media. However, rising prices and increasing demand for these sugars indicate the necessary exploitation of alternative, carbon rich substrates of lower market value that are available in sufficient amount in order to reduce xanthan production costs caused by high price of glucose and sucrose [10].

Recent research related to xanthan production has been focused on the possibility of use of substrates that meet aforementioned criteria, such as sugarcane molasses, whey, glycerol, kitchen waste and olive mill wastewaters [11]. Among examined substrates, glycerol proved to be one of the most promising for xanthan biosynthesis. The aim of this review study is to discuss data from available scientific literature related to the possibility of xanthan production on a glycerol-based media using different *Xanthomonas* strains.

Material

The available scientific publications were used as a primary material for this paper. The collated data were selected, systematized, compared and critically discussed.

Discussion

Research related to the development of a biotechnological process of the production of xanthan on glycerol-based media are new and still in initial stages, due to impaired metabolic activity of the applied producing microorganism [12]. Only a few studies focused on xanthan production on a medium containing commercial glycerol have been reported. This study is focused on research with commercial glycerol so that it could potentially be replaced by cheaper raw material, crude glycerol, the main by-product of the biodiesel industry. As the demand and production of biodiesel grow exponentially, the utilization of the glycerol becomes an urgent topic [13].

First study focused on the xanthan production on glycerol-based media is the study of Serbian research team where the effect of different initial glycerol concentrations in the medium on the success of biopolymer biosynthesis by X. campestris ATCC 13951 was examined [14]. In this study, commercial glycerol in concentrations of 10.00 g/L, 20.00 g/L, 30.00 g/L, 40.00 g/L, 50.0 g/L and 60.00 g/L was added to the production media. The biosynthesis was carried out in 300 mL Erlenmeyer flasks (100 mL working volume) in batch mode under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 168 h. The results obtained in applied experimental conditions suggest that xanthan production by cultivation of reference strain on all six media was successful. The values of xanthan concentration in media at the end of bioprocess indicate that the biosynthesis of the desired biopolymer increased with the increase in the initial glycerol concentration in the medium. The highest xanthan concentration of 15.81 g/L was obtained in the media with a glycerol content of 60.00 g/L and the lowest xanthan concentration of 9.82 g/L was obtained in the media with a glycerol content of 10.00 g/L. The average molecular weights of the xanthan biosynthesized in this research ranged from $2.64 \cdot 10^5$ Da to $3.17 \cdot 10^5$ Da. According to the obtained results there is no significant difference in the quality of the produced xanthan and it is concluded that different initial glycerol concentrations in the cultivation medium do not significantly affect the quality of the biopolymer under the applied experimental conditions. The results obtained in this study were the basis for development of xanthan production on glycerol-based media in order to increase the xanthan yield and its quality.

After the confirmation that xanthan production on medium containing commercial glycerol as a sole carbon source by reference strain X. campestris ATCC 13951 is possible, previously mentioned researchers conducted another investigation to compare the producibility of X. campestris strains isolated from the environment with the reference strain [15]. In this research, xanthan biosynthesis was carried out by cultivation of reference strain and eight strains isolated from the infected leaves of several different cruciferous plants, such as cabbage (I2, I4, I6, I8), kale (I1, I7) and cauliflower (I3, I5) on glycerol-based medium in 300 mL Erlenmeyer flasks (100 mL working volume) in batch mode under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 120 h. The obtained results show that the xanthan production in applied experimental conditions was influenced by the used strain. Xanthan concentration in media at the end of performed bioprocesses was in the range from 1.68 g/L to 7.24 g/L, while the highest values of this parameter was obtained when reference strain was used. The findings of this study indicate that glycerol is appropriate carbon source for xanthan biosynthesis in the applied experimental conditions by all tested Xanthomonas strains. The authors concluded that further research should be focused on improvement of production process in order to increase the bioprocess efficiency.

Results from research performed in China indicate that xanthan can be produced by a mutant strain *X. campestris* CCTCC M2015714 on medium with glycerol as the sole carbon source [16]. In this study, xanthan was produced on medium with 40 g/L commercial glycerol. The bioprocess was performed in a 7 L bioreactor (4 L working volume) under aerobic conditions at temperature of 30°C and agitation rate of 400 rpm for 84 h. Characterization of xanthan biosynthesized in applied experimental conditions through FT-IR and NMR resulted in conclusion that chemical structure of produced xanthan is similar to that of the commercial xanthan. The molecular weight of xanthan produced by mutant strain *X. campestris* CCTCC M2015714 on glycerol-based medium was $3.0\pm0.14\cdot10^6$ Da. The results obtained in this study suggest that xanthan produced in applied experimental conditions could be used as dietary fiber potentially in food industry because of the low viscosity.

Optimization of a glycerol-based medium for xanthan production by X. campestris ATCC 13951 was performed in 2020 in Serbia [17]. The xanthan biosynthesis was carried out simultaneously in 300 mL Erlenmeyer flasks (100 mL working volume) in batch mode under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 168 h. Glycerol content in cultivation medium was varied from 15 g/L to 45 g/L. Response surface methodology was used for the optimization of this bioprocess. The results of optimization obtained in this research suggest that the maximal xanthan concentration from 10.71 g/L to 11.25 g/L can be achieved when biosynthesis is performed by cultivation of producing strain in media containing glycerol in concentration of 32.96 g/L. The average molecular weight of the xanthan biosynthesized in applied experimental conditions is in range from $3.28 \cdot 10^5$ Da to $3.32 \cdot 10^5$ Da. According to the authors, commercial glycerol represents an appropriate replacement for traditionally used carbon sources responsible for quantity and quality of the biopolymer. Additionally, it is proven that commercial glycerol can be substituted with crude glycerol generated by a biodiesel industry, as a cheap alternative substrate, but further research is necessary in order to determine the optimal process parameters for xanthan biosynthesis and to examine the success of application of novel producing strains.

Recent study focused on the possibility of using glycerol as substrate for xanthan production by different Xanthomonas strains was performed also in Serbia [18]. The reference strain X. campestris ATCC 13951, eight Xanthomonas strains isolated from crucifers and five Xanthomonas strains isolated from pepper leaves were used as producing microorganisms in this research. Xanthan production was carried out in 300 mL Erlenmeyer flasks (100 mL working volume) under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 168 h. The obtained results indicate that xanthan production in applied experimental conditions was possible by all examined strains. According to the determined biopolymer quantity, xanthan concentration varied from 5 g/L to 7 g/L when biosynthesis was performed by Xanthomonas strains isolated from crucifers and from 8 g/L to 10 g/L when Xanthomonas strains isolated from pepper leaves were used. This indicates that glycerol potentially may be suitable carbon source for industrial xanthan production by Xanthomonas strains isolated from pepper leaves. On the other side, the molecular weight of xanthan produced in applied experimental conditions varied from $2 \cdot 10^5$ Da to $5 \cdot 10^5$ Da when *Xanthomonas* strains isolated from crucifers was used and from $5 \cdot 10^5$ Da to $8 \cdot 10^5$ Da when *Xanthomonas* strains isolated from pepper leaves was producing microorganisms. The obtained results suggest that glycerol is suitable carbon source not only for the xanthan production in large quantities but also for the biosynthesis of good-quality biopolymer. It is concluded that Xanthomonas strains isolated from pepper leaves have the greatest potential for application in biotechnological production of xanthan on glycerol-based medium.

Conclusion

This paper provides valuable information about the possibility of biotechnological xanthan production on a glycerol-based medium by different *Xanthomonas* strains. According to the results discussed above, glycerol has great potential for the efficient production of xanthan, but in order to achieve successful xanthan biosynthesis it is necessary to select appropriate *Xanthomonas* strains. Data presented in this review study can be useful for the selection of producing strain, formulation of medium composition and optimization of process conditions for successful xanthan production on glycerol-based medium. Results presented in this research may be also a suitable background for future investigations and development of the economically justified production of xanthan on glycerol-based medium.

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CRITERIA FOR EVALUATION OF WASTE GLASS/CLAY BLOCKS PRODUCTION MODEL

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Abstract

One of the ways to solve the problem of waste mixed packaging glass recycling is to use glass packaging as a secondary raw material to obtain a new product - a block of clay with a certain mass fraction of crushed glass. The paper describes the criteria that are very useful for the analysis of the impact on the environment and the analysis of the economic factor when applying the model of the use of waste glass packaging, as a secondary resource in the production of clay blocks.

Introduction

Environmental natural resources are transported and used as raw materials in production plants, turning them into construction products, which further generates pollution and requires significant energy consumption with appropriate greenhouse gas emissions. The choice of construction products with less impact on the environment is one of the ways to reduce the negative impact of construction on the environment. Therefore, the environmental impact assessment must be balanced with the economic assessment. To satisfy their customers, manufacturers and designers must develop and select construction products with a reasonable balance between the results of environmental performance (Environmental Performance Score) and the result of economic performance (Economic Performance Score), which is not an easy task. To this end, the US National Institute of Standards and Technology (NIST) has developed BEES (Building for Environmental and Economic Sustainability - BEES) software that can be used as a tool to assess sustainability in the domain of environmental protection and economic sustainability in the use of certain construction materials [1].

The paper will present the criteria that were taken into account when assessing the impact of clay blocks production with a certain mass fraction of recycled waste glass on the environment.

Experimental

The BEES methodology quantifies the environmental impacts of construction products using a multidisciplinary approach known as Life cycle assessment (LCA). This means that it takes into account the multiple environmental and economic impacts during the life of a particular construction product. Consideration of multiple impacts and life cycle stages is necessary because product selection decisions based on individual impacts or phases may exclude some impacts that may cause equal or greater damage. In other words, a multidimensional life cycle approach is needed for a comprehensive, balanced analysis [2].

The LCA is a systematic approach to environmental impact assessment standardized by the International Standards Organization (ISO) [3]. In particular, BEES determines the overall impact of construction products on the environment using the life cycle assessment approach

specified in the ISO 14040 series standards [4]. All phases in the life of a certain product are analyzed: procurement of raw materials, production, transport, use, recycling and waste management. To determine economic performance, the Life Cycle Cost Method is used, which includes the costs of initial investment, replacement, production processes, maintenance and repair and disposal [2].

The general objectives of waste management, and thus waste glass packaging, relate to the protection of human health and environmental quality, resource conservation and sustainability [5], [6].

Results and discussion

Taking into account the approach of BEES methodology applied in the model of production of ceramic tiles with glass recycling [1] and general objectives of waste management, the paper defines the criteria for evaluation of the glass / clay blocks production model (Figure 1).





Within the evaluation of the model, two groups of criteria were considered: Environmental Impact Criteria and Economic Criteria. The basic criteria are: Preservation of resources with an emphasis on the preservation of space required for disposal, preservation of natural resources, in this example clay, and the recycling rate which refers to increasing the percentage of waste glass packaging recycling. The economic factor must also be taken into account, i.e. the calculation of the production price of commercial blocks and blocks with a mass share of recycled glass in its composition.

Conclusion

Hodge et al. (2010) in the research emphasized the importance of investing efforts in order to increase the use of waste materials, as ancillary resources of primary resources in industry,

considered it an important step towards sustainable industrial development [7]. In the mentioned research, a new approach of recycling application within the boundaries of industry is presented, with a focus on the whole industry, in order to identify locations where there is a benefit both from the economic aspect and from the aspect of environmental protection.

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