

Photoinduced processes in homogenous and heterogenous systems studied by EPR spectroscopy

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Photoinduced processes & EPR



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Reactive Oxygen Species (ROS)

<u>Reactive Oxygen Species (ROS) – generally small highly reactive and non-persistent species – presence of unpaired valence shell electrons</u>
<u>superoxide radical anion/hydroperoxide radical O2*/HOO*</u>
<u>hydroxyl radical *OH</u>
<u>singlet oxygen 'O2</u>
<u>hydrogen peroxide H2O2</u>

Photoinduced production of superoxide radical anions (Type I photo-oxidation) or singlet oxygen (Type II photooxidation) initiated by light observed for alkaloids, natural quinones and other substances.

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Type I – electron transfer

A^{*}(T_{1}) + R \rightarrow A^{\bullet-} + R^{\bullet+}

A^{\bullet-} + {}^{3}O_{2} \rightarrow A + O_{2}^{\bullet-}

O_{2}^{\bullet-} + R \rightarrow R_{ox}
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$$\mathsf{A}^*(\mathsf{T}_1) + {}^3\mathsf{O}_2 \to \mathsf{A}^{\bullet +} + \mathsf{O}_2^{\bullet -}$$

<u>Type II – energy transfer</u> $A^{*}(T_{1}) + {}^{3}O_{2} \rightarrow A(S_{0}) + {}^{1}O_{2}$ STU



Photocatalysis – excitation of semiconductor particles



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$$e^{-} + O_{2} \rightarrow O_{2}^{\bullet-} \qquad h^{+} + H_{2}O \rightarrow \bullet OH + H^{+}$$

$$H^{+} + O_{2}^{\bullet-} \rightarrow \bullet O_{2}H \qquad h^{+} + HO^{-} \rightarrow \bullet OH$$

$$2 \bullet O_{2}H \rightarrow H_{2}O_{2} + O_{2}$$

$$e^{-} + H_{2}O_{2} \rightarrow \bullet OH + HO^{-} \qquad HO^{\bullet} + R \rightarrow \text{intermediates} \rightarrow CO_{2} + H_{2}O$$

H+

Photoinduced proccesses

???

Radical intermediates

formed upon UV or visible light exposure in **homogeneous** and **heterogeneous** systems

??? Method of detection

of non-persistent radical intermediates

Redox reactions – associated with the production of

radical intermediates

Electron Paramagnetic Resonance (EPR)

spectroscopy

a unique tool in detection and identification of radical intermediates



What is EPR ?

Electron Paramagnetic Resonance spectroscopy is the only spectroscopic technique that provides direct detection of free radicals and other samples that contain unpaired electrons



EPR – studied systems



How does EPR work?



A peak in the absorption occurs when the energy difference between two levels $(g_e \mu_B B)$ matches the energy of irradiation (hv)

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How does EPR work?



1. derivation

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In cw-EPR experiments we keep the radiation frequency constant and scan the magnetic field EPR spectrum is recorded as its 1. derivation and is characterized by g-factor and hyperfine splitting constants (hffc) a_i

Multiplet structure

- Hyperfine interactions (hfcc; with the nuclear spin of the "host" atom or metal ion)
- Zero-field interactions
- Dipole-dipole interactions (with other electron spins existing in the sample)

Hyperfine interaction splits EPR line into (2nl+1) lines

I – nuclear spin, n – number of equivalent nuclei



EPR spectra of atoms and molecules



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g-value/factor – a "fingerprint" of the radical; characteristic of the local environment around the paramagnet and generally depends on the orientation of the molecule with respect to the vector of *B* (information about a paramagnetic center's electronic structure, electronic structure and symmetry)



EPR spectra of atoms and molecules

Quantity in EPR spectroscopy (concentration)

The concentration of photogenerated paramagnetic species – evaluation from the double-integrated EPR spectra based on the calibration curve obtained from the EPR spectra of semi-stable free radicals with known concentration.

All spectra must be measured under the strictly identical experimental conditions.







EPR spectrometer

The g-factor is a constant but the field for resonance changes with microwave frequency

| Microwave Band | Microwave Frequency (GHz) | B (for g = 2) Gauss (= 1·10 ⁻⁴ Tesla) |
|-------------------|------------------------------|---|
| L | 1.1 | 392 |
| S | 3.0 | 1070 |
| Х | 9.5 | 3389 |
| К | 24.0 | 8560 |
| Q | 35.0 | 12485 |
| W | 94.0 | 33600 |

In cw-EPR experiments we keep the radiation frequency constant and scan the magnetic field

EPR spectrometers and irradiation sources



standard cw-EPR spectrometers Bruker EMX and EMX Plus X- a Q-band coupled with temperature unit

(77 K - 333 K)

EPR flat quartz cells





EPR spectrometers and irradiation sources



Photoinduced processes are conducted with the production of **free radicals**



Majority of free radicals are short-lived species – direct detection via cw-EPR is not possible

EPR spin trapping technique – method of indirect detection and identification of non-persistent free radicals

Trapping of reactive short-lived free **radicals (R)** by a diamagnetic **spin trap (ST)** compound *via* addition reaction producing more stable paramagnetic free radical product – **spin adduct**





indirect information on the trapped radical

Spin-adduct formation



Genuine spin trapping

 $ST + {}^{\bullet}R \rightarrow {}^{\bullet}ST-R$

Inverted spin trapping

 $ST - e^- \rightarrow ST^{*+} + R^- \rightarrow *ST-R$

Forrester-Hepburn mechanism

 $ST + R^- \rightarrow ST-R(H) - e^- \rightarrow *ST-R$



An ideal spin trapping agent:

- should be stable under reaction conditions
- should not participate in any side reactions leading to paramagnetic species
- should undergo radical reaction very quickly
- should lead to spin-adducts characterized with relatively simple EPR spectra
- should provide the largest set of structural information





Spin trapping agents



Spin traps and schemes of formation of spin adducts



СНРТ

<u>Nitroso</u> spin trapping agents react with free radical species *via* **nitrogen** located in α -position relative to free electron

Titanium dioxide TiO₂













O. Ola, M. M. Maroto-Valer, J. Photochem. Photobiol C Rev. 24 (2015) 16–42.

TiO₂ applications



TiO₂ nanostructures – increasing popularity due to their attractive electrical, mechanical and optical properties which makes them low cost, non-toxic and durable alternative for large number of applications



Two main approaches to expand the photocatalytic activity

- 1. modification of the electronic and optical properties of the photocatalyst by metal or non-metal doping or by combination with another semiconductor (composite semiconductors)
- 2. control of the microstructure and morphology of TiO₂ (photoinduced processes are significantly influenced by the crystal structure of the photocatalyst, particle size, their morphology and porosity)

Radical intermediates formed upon UV and visible exposure





Formation of •DMPO-OH

? genuine spin trapping (reaction of the hydroxyl radicals with DMPO)
? inverted spin trapping via DMPO⁺⁺ with subsequent nucleophilic attack of water molecules
? decomposition of unstable •DMPO-O₂H from the photogenerated O₂•-/•O₂H

Source of reactive species (adsorbed H₂O/OH⁻ or lattice oxygen)



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generation of the surface hydroxyl radicals - addition of DMSO to the TiO₂/H₂O system: rapid reaction of HO[•] with DMSO - production of methyl radicals





Problems with superoxide detection in water

- 1. Superoxide is stable in aprotic solvents; rapid dismutation in water
- 2. Low value of rate constants for the addition of $O_2^{\bullet-}/O_2H$ to the N-oxide spin traps (e. g. for DMPO $k = 6.6 \times 10^3 \text{ M}^{-1} \text{ s}^{-1} \text{ pH} \le 5;$ $k = 1.0 \times 10 \text{ M}^{-1} \text{ s}^{-1} \text{ pH} \ge 7.8$)
- 3. Low stability of •DMPO-O₂^{-/}O₂H spin-adduct ($\tau_{0.5} = 59$ s); interconversion of •DMPO-O₂^{-/}O₂H to •DMPO-OH!!!

Selected ROS pathways in aqueous titania photocatalytic systems

new generation N-oxide spin traps (DIPPMPO, EMPO) – especially designed for detection of superoxide radical anion in aqueous media and biological systems - improved stability of •ST-O₂/O₂H

ST+ •OH \rightarrow •ST-OH or ST + O₂•-/O₂H \rightarrow •ST- O₂-/O₂H



no 'ST-O₂'/**O**₂**H formation** O_2^{-} is preferably transformed to H_2O_2 (disproportionation with protons)



Dvoranová, D., et al. Molecules 2014, J19, 17279-17304



Solvent properties – influence on the generation of electron-hole pairs upon exposure and their consecutive reactions



determined in aqueous solutions.

Spin trapping in DMSO suspension



Spin trapping in alcohol suspensions



Spin trapping in alcohol suspensions



Spin trapping in ACN suspension



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EPR methods used for testing on photoactivity:

- **Spin trapping** using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) spin trapping agent in aqueous/DMSO suspensions in the presence of molecular oxygen
- **Spin scavenging** (stable radical (TEMPO derivatives) reacts with the reactive free radical to form a diamagnetic product (Tempo-adduct)
- Elimination of semi-stable free radicals (termination of radical cation ABTS*+ in aqueous suspensions in the presence of molecular oxygen
- Oxidation of sterically hindered amines (SHA) to nitroxide radicals in dry acetonitrile suspensions under inert atmosphere (oxidation of diamagnetic TMPO to paramagnetic Tempone)



Tested samples

TiO₂

ANATASE nanocrystals with exposed {001} crystal facets prepared by solvothermal method using HF as capping agent Undoped and doped with Mg²⁺ ions (**2 at %, 5.1 at % and 6.2 at %)**

as-prepared washed calcinated (residual F⁻) NaOH 600 °C



M.-V. Sofianou et al. Appl. Catal. B. 142– 143 (2013),761–

2D-TiO₂nanosheets prepared by lyophilization and subsequent annealing (500 °C, 650 °C, 800 °C, 950 °C) modified with Nd, Au and Ag (1 at %)



E. Pližingrová et al. Catal .Today (2016) in press

Composite photocatalysts TiO₂/**g-C**₃**N**₄ (graphitic carbon nitride) prepared by annealing of melamine and dry mixtures of melamine and TiO₂ P25 at 550 °C (melamine/TiO₂ wt. ratio **M1** (3:1), **M2** (1:1), **M3** (2:3), **M4** (1:3), **M5** (1:4), **M6** (1:7))



T. Giannakopoulou et al. Chem. Eng. J. (2016) in press



Tested samples



- organic polymer-like material with 2D structure similar to graphene
- × insoluble in water
- x excellent thermal (up to 600 °C in air) and chemical (stable in highly acidic and alkaline media) stability due to strong covalent bond between carbon and nitrogen atoms
- visible-light active semiconducting material with band-gap ~ 2.7 eV/460 nm

Graphitic carbon nitride $(g-C_3N_4)$

Heptazine (tri-s-triazine) based structure model of $g-C_3N_4$





(Hi)Story of g-C₃N₄

μέλλον = future (Greek)





3

0

M1

Μ4

М3

M2

M5

P25

M6

calibration curve obtained from the EPR spectra of Tempol solutions measured under the strictly identical experimental conditions

Z. Barbieriková et al. J. Catal. 331 (2015) 39-48



Stability of spin-adducts in aqueous media MCN-TE/air/water/365 nm





Spin scavenging with TEMPO derivatives

Stable radical (TEMPO derivatives) reacts with the reactive free radical to form a diamagnetic product (Tempo-adduct)



CN series Tempone/water:DMSO(4:1 vol./air)



MCN series Tempol/water:DMSO(1:4 vol./air)





 $c(photo) = 0.8 \text{ mg} \cdot \text{mL}^{-1}, c_0(Tempol) = 10 \ \mu\text{M})$

Elimination of semi-stable free radicals

termination of radical cation ABTS^{•+} (2,2'-azino-bis(3-ethylbenzo-thia-zoline-6-sulphonate) via oxidation to diamagnetic azodication ABTS²⁺ (absorption maxima at 260 and 294 nm) via reduction to diamagnetic parent molecule ABTS (absorption maxima at 225 and 343 nm)



Elimination of semi-stable free radicals



Elimination of ABTS⁺⁺ $g-C_3N_4/ABTS^{++} \xrightarrow{\lambda_{max} = 365 \text{ nm}} g-C_3N_4/ABTS$



aerated suspensions continuous irradiation $\lambda_{max} = 365 \text{ nm}$ EPR

water/DMSO (4:1, vol.)









UV/VIS spectra monitored in situ upon continuous UVA exposure (diode array UV/VIS/NIR spectrophotometer connected with KEVA photoreactor)

c(photo) = 0.333 g L⁻¹; c_0 (ABTS^{•+}) = 58 μ M UVA irradiance~15 mW

Reduction of ABTS $^{\bullet +}$ radical cation by photogenerated electrons and $O_2 ^{\bullet -}$

(standard reduction potential in aqueous media for ABTS $^{\star+}/\text{ABTS}$ +0.68 V vs. NHE)

$ABTS^{+} + e^{-} \rightarrow ABTS$

 $O_2 + e^- \rightarrow O_2^{\bullet -}$ ABTS^{•+} + $O_2^{\bullet -} \rightarrow ABTS + O_2$





Oxidation of sterically hindered amines

oxidation of sterically hindered amines to nitroxide radicals in dry acetonitrile suspensions under inert atmosphere e.g. oxidation of diamagnetic TMPO (4-0x0-2,2,6,6-tetramethylpiperidine) to paramagnetic Tempone (4-0x0-2,2,6,6-tetramethylpiperidine N-oxyl)



12 mW cm⁻²

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Oxidation of sterically hindered amines



Schematic overview of reactive species generation upon photoexcitation of titanium dioxide nanoparticles in the oxygenated and deoxygenated acetonitrile suspensions.

Oxidation of sterically hindered amines

TiO₂ / TMPO / AgNO₃ / ACN / argon ZnO / TMPO / AgNO₃ / ACN / argon



TMPO adsorption on the surface

adsorption of Ag(I) ions (reaction with electrons – silver metal deposition on titania surface



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Topoisomerase poisons

OH /

 H_5C_2

H_cC

C,H₅

Role of anticancer drugs – destructions of cancer cells

Topoisomerase – enzymes involved in the replication of DNA

- Topo I makes single-strand breaks
- Topo II makes double-strand breaks



Camptothecin

H₋C

C,H5

SN-38

- the first described inhibitor of Topo I
- quinoline pentacyclic plant alkaloid from tree Camptotheca acuminata ("xi-shu" – happy tree)
 - presence of carboxylate and lactone form
- lactone biological active, carboxylate toxic
 - Camptothecin (CPT) low solubility in water
- Irinotecan (CPT-11) and Topotecan are the only Topo I inhibitors used in the clinic.

HO

 CPT-11 – pro-drug converted by decarboxylation to active and more cytotoxic metabolite SN-38

carboxylesterase

Photoexcitation of **CPT-11**/DMSO/air or CPT-11/DMSO/water/air solutions in the presence of DMPO spin trapping agent



Presence of SOD – decrease of • DMPO-O,⁻

Photoexcitation of **SN-38**/DMSO/air or **SN-38:Cu**²⁺/DMSO/air solutions in the presence of DMPO spin trapping agent



Magnetic sweep width was 10 mT, initial concentration of SN-38 and Cu²⁺ was 0.8 mM and initial concentration of DMPO was 42.4 mM. Irradiation of SN-38: domination of ROS Irradiation of SN-38:Cu²⁺: formation of 'OCH₃ suppressed

Photoexcitation of **SN-38** and **SN-38:Cu**²⁺ in DMSO and DMSO-d6 under argon in the presence of ND



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Systems were irradiated 10 minutes Accumulation of EPR spectra started at 5th minute of irradiation

Photoexcitation of **CPT-11** and **CPT-11**:Cu²⁺ in aqueous solutions in the presence of POBN



Photoexcitation of **CPT-11** and **CPT-11**:Cu²⁺ in aqueous solutions in the presence of POBN



Photoexcitaion of CPT-11 a CPT-11:metal ion (Cu²⁺, Co²⁺, Fe³⁺) in the presence of TMP selective reagent for detection of singlet oxygen in ACN



Generation of 'O,

CPT-11:Co²⁺ **CPT-11:Cu²** CPT-11:Fe³ high fails and folgers and folgers and folgers and CPT-11 0.0 4.5 9.0 13.5 Irradiation time, min

Series of EPR spectra (SW 6 mT) measured upon continuous irradiation of CPT-11 and equimolar CPT-11:metal ion in ACN int he presenece of TMP..



Conclusions

The EPR spectroscopy techniques represent valuable tools for the characterization of radical intermediates generated in irradiated homogeneous and heterogeneous systems.

Careful selection of the experimental conditions and a precise analysis of the experimental EPR spectra considering alternative reaction pathways is an important aspect of any successful application of the indirect techniques in the characterization of photoactivity of photocatalysts.