Kinetic investigation of the decomposition of drugs

Theoretical background: P.W. Atkins: *Physical Chemistry*, 26.1–3 (4th Edition), or 25.1–3 (6th Edition) chapters.
Type of practice: Pairwise.
Aim of practice: To determine the rate of decomposition of one of the most prevalent analgesics, acetylsalicylic acid[1] in aqueous solution. In Hungary, it is found in medicaments with brand names such as *Kalmopyrin, Istopirin*, and *Aspirin*, but more than 200 patented pharmaceutical products are available worldwide which contain this drug.

1 Introduction

The bark of birch species has already been used in the antiquity for the relief of pain and fever. Many centuries later, ascorbic acid turned out to be the pharmaceutically active component (i.e., drug), although its sustained or high-dose administration can cause acute stomach pain and ulcer. At the end of the 19th century, experiments revealed that the side effects can largely be mitigated when the direct use of salicylic acid (SA) is avoided, and it is converted to ester by acetic acid (AcOH). Acetylsalicylic acid (AcO-SA) is a chemically stable compound, which can be stored in solid form for years under ambient conditions. However, it is unstable in aqueous solutions. It reacts with water and slowly (within hours) it is decomposed to salicylic acid and acetic acid according to the equation shown in Figure 1.

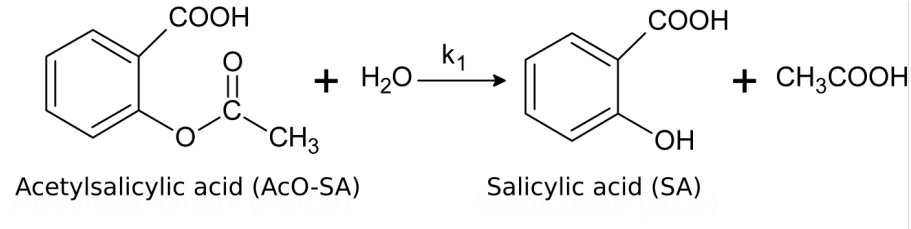

Figure 1: Decomposition of acetylsalicylic acid in aqueous medium.

If this slow hydrolysis occurs in the stomach, it sustains a salicylic acid concentration, which can suppress fever and pain, but it is low enough to assure a considerable reduction of side effects.

The hydrolysis proceeds much faster in alkaline solutions, where the reaction partner is the hydroxide anion next to / instead of water. In strongly alkaline solutions ($p\mathrm{H} > 13$), hydrolysis is completed within an hour, therefore it is recommended to determine the total drug content under such conditions.

During the laboratory practice, the process of hydrolysis is investigated under slightly alkaline conditions and at temperature above that of the human body, in order to complete the experiments within the time limitations. In the human body, hydrolysis proceeds under acidic conditions and it is catalyzed not only by the gastric acid, but by enzymes as well. In *real-life application*, hydrolysis proceeds somewhat more slowly than under the condition of this lab practice.

2 Relevant equations

The reaction in water can be considered pseudo-first-order in regard to acetylsalicylic acid (AcO-SA), because of the large excess of water and the constant hydroxide ion concentration (since the stoichiometric equation tells us that the latter is not consumed at all). Consequently, the integrated form of the first-order kinetic equation describes the concentration of AcO-SA in time:

$$[\text{AcO-SA}]_t = [\text{AcO-SA}]_0 \cdot e^{-k \cdot t} \qquad (k = k_2 \cdot [H_2O]), \tag{1}$$

[1] Its IUPAC name is 2-acethoxy benzoic acid, sometimes – incorrectly – it is referred to as aspirin.

where k is the pseudo-first-order rate constant.

The SA yielded by the reaction forms purple and stable complex with Fe(III) ions, which can be quantitatively detected in the visible spectrum by a spectrophotometer. Using sufficiently high concentrations of Fe(III) and adequate *p*H, the total amount of SA can be brought into complex form in which the metal ion and the ligand (i.e. salicylate ion) are in equimolar amount (1:1 molar ratio). Consequently, in terms of the Beer–Lambert law, the absorbance measured at λ wavelength in at time t (A_t^λ) will be proportional to the concentration of SA:

$$\mathrm{A}_\mathrm{t}^\lambda = \varepsilon_\mathrm{complex}^\lambda \cdot [\mathrm{complex}]_\mathrm{t} \cdot \text{ł} = \varepsilon_\mathrm{complex}^\lambda \cdot [\mathrm{SA}]_\mathrm{t} \cdot \text{ł}, \tag{2}$$

where $\varepsilon_\mathrm{complex}^\lambda$ is the molar absorbance[2] of the iron(III) salicylate complex at the applied wavelength and ł is the optical path length (usually 1 cm). Since concentrations are often determined photometrically by using only one specific wavelength (usually at or near the peak maximum), the notation of the wavelength will be omitted in the following for the sake of simplicity.

It is also worth transforming Equation (1) so that it will contain only the concentrations of SA, because we can only monitor the light absorption of this hydrolysis product. In terms of reaction stoichiometry (see Figure 1), the initial concentration of AcO-SA equals to the final (equilibrium) concentration of SA:

$$[\mathrm{AcO\text{-}SA}]_0 = [\mathrm{SA}]_\infty .$$

Moreover, it is also valid that, in any moment of the reaction, the concentration of unreacted acetylsalicylic acid can be described as

$$[\mathrm{AcO\text{-}SA}]_\mathrm{t} = [\mathrm{SA}]_\infty - [\mathrm{SA}]_\mathrm{t} .$$

Substituting these relationships into Equation (1) reads as

$$[\mathrm{SA}]_\infty - [\mathrm{SA}]_\mathrm{t} = [\mathrm{SA}]_\infty \cdot \mathrm{e}^{-\mathrm{k}\cdot\mathrm{t}} .$$

By substituting concentrations to absorbances using Equation (2) and then taking the natural logarithm, we get the formula that is appropriate for the evaluation of the experimental data:

$$\boxed{\ln(\mathrm{A}_\infty - \mathrm{A}_\mathrm{t}) = \ln \mathrm{A}_\infty - \mathrm{k}\cdot\mathrm{t}} . \tag{3}$$

Based on this equation, the rate constant of the reaction can be directly determined from the measured absorbances, and A_∞ is useful to judge the precision of the measurement. It is also obvious from Equation (3) that it is not necessary to determine the concentrations of either the drug or its decomposition product. The determination of the kinetic parameters only requires the knowledge of the temporal change of the absorbance (which is proportional to concentration).

Although the rate constant characterizes a pseudo first order reaction, the half-life $\left(\mathrm{t}_{1/2}\right)$ is often used as well. This is a time interval during which the initial concentration of the reactant decreases to its half[3]. There is a direct correlation between the first-order rate constant and the half-life, which can be easily derived by means of Equation (1). When the half of the amount of the reactant is consumed, $[\mathrm{AcO\text{-}SA}]_\mathrm{t} = [\mathrm{AcO\text{-}SA}]_0/2$. Substituting this into Equation (1) and expressing time leads to

$$\boxed{\mathrm{t}_{1/2} = \frac{\ln 2}{\mathrm{k}}} . \tag{4}$$

If rate constants are known at different temperatures, both the activation energy of the reaction ($\mathrm{E_a}$) and the pre-exponential factor $\mathbf{A}$[4] can be calculated via the Arrhenius equation (R is the universal gas constant):

$$\mathrm{k_T} = \mathbf{A} \cdot \mathrm{e}^{-\mathrm{E_a}/(\mathrm{R}\cdot\mathrm{T})} .$$

[2] In general, when not only light absorption but scattering and reflection also contribute to the decrease of the transmitted light intensity, it is rather called *molar attenuation coefficient*.

[3] This definition is unambiguous for a first order reaction, but not for more reactants. In the latter case, it refers to the reactant which – considering the stoichiometry of the reaction – is present in the lowest amount.

[4] In the literature, both the absorbance and the pre-exponential factor is noted with A. To avoid confusion, in this description we write the pre-exponential factor in bold ($\mathbf{A}$).

By determining the rate constants (k_{T_1} and k_{T_2}) at two different temperatures (T_1 and T_2), the following expressions are obtained for E_a and $\mathbf{A}$ (see also note 2 at the end of the description):

$$\boxed{E_a = \frac{R}{\frac{1}{T_2} - \frac{1}{T_1}} \cdot \ln \frac{k_{T_1}}{k_{T_2}}} \text{ and } \boxed{\mathbf{A} = \frac{(k_{T_1})^{(T_1/(T_1-T_2))}}{(k_{T_2})^{(T_2/(T_1-T_2))}} \qquad \left(= k_T \cdot e^{E_a/(R \cdot T)}\right)}. \tag{5}$$

3 Instructions

In the course of the practice, students are going to investigate the decomposition of acetylsalicylic acid content of a pill at two different temperatures. The applicable temperatures are assigned by the teacher between 38 – 43 °C and 53 – 58 °C. If not stated otherwise, these temperatures are 40 and 55 °C. Samples must be withdrawn from the reaction mixture at predetermined times. Transferm the SA content into its Fe(III) complex and measure the absorbance of the solutions at 552 nm. Unless stated otherwise, the approximate times of sample collection are 10, 20, 30, 40, 50, 60, and 70 minutes after the initiation of the reaction.

Set the thermostat to the lower temperature. While the thermostat reaches steady temperature, 50 cm^3 of HCO_3^- / CO_3^{2-} buffer must be prepared, the *p*H of which is 9.7 – 10.3 and the measured mass of Na_2CO_3 falls between 0.5 – 1.0 g to maintain the necessary buffer capacity. Exact values will be given by the instructor. Calculate the mass of the chemicals to be weighted, if the *p*H of the buffer depends on the concentration of the acid and conjugate base according to the Henderson–Hasselbalch equation

$$p\mathrm{H} = p\mathrm{K_a} + \lg\left(\frac{[\text{conjugate base}]}{[\text{weak acid}]}\right),$$

where $K_a (= 4.688 \times 10^{-11})$ is the dissociation constant of the acid and the square brackets represent total concentrations calculated from weighting. Weight both salts into the same beaker of 25 – 100 cm^3, and dissolve them in an amount of water as low as possible. Wash-transfer these into a 50 cm^3 volumetric flask and fill up to level by water.

The part of the pill containing ca. 100 mg drug must be carefully powdered and dissolved in a small amount of water, so that the total volume is between 16 – 19 cm^3. The dissolution of acetylsalicylic acid is a slow process; the mixture needs to be stirred by a glass rod. The solid content of the tablet does not dissolve completely. Therefore, the solution must be filtered via a filter paper into a 25 cm^3 volumetric flask, so that the insoluble part would not cause an artefact during the absorbance measurement[5].

When the thermostat reached the preset temperature, immerse the buffer and drug stock solutions into it for 10 – 15 minutes. The setting of uniform temperature distribution in the solutions can be accelerated if they are shaken in every 3 – 4 minutes. While the stock solutions are being thermostated, students should prepare *quenching* solutions to determination how much AcO-SA decomposed until time *t*; the procedure is the following: pipet exactly *(1)* 0.50 cm^3 0.25 M HCl solution and *(2)* 3.0 cm^3 0.10 M $FeCl_3$ solution into all of the 7 pieces of 25 cm^3 volumetric flasks. The flasks are then filled to half-volume by purified water (*no tap water*) and placed into a bowl of water–ice mixture (see also the first note at the end of the description).

Prepare also the solution needed for the determination of the total AcO-SA content of the stock solution! Pipet 0.3 cm^3 of 0.25 M NaOH solution into a 100 cm^3 volumetric flask and place it as well into the thermostat.

When the stock solutions reached to assigned temperature, add (by a pipette) 5.0 cm^3 buffer solution into the drug stock solution and *homogenize it*. Simultaneously to the addition of the buffer, start the timer; this event is the starting time of the reaction. The volumetric flask does not need to be filled up to level because the evaluation process relies only on the relative drug content. Yet, at least 20 – 22 cm^3 volume is required for the measurements!

[5]Certainly, a small amount of drug is retained by the filter paper and on the wall of the beaker, but the concentration of the stock solution does not alter the pseudo first-order measurement thus this loss causes no error.

Directly afterwards, take 2.0 cm^3 sample (*without removing the solution from the thermostat!*) and add it to the 100 cm^3 flask containing the basic solution. The sample fraction adhered to the side wall of the flask should be washed into the basic solution completely, using ca. 2 – 3 cm^3 purified water. Until the end of the measurement, for ca. 70 minutes, leave this flask in the thermostat and unfilled to its level.

Next, take 2.00 cm^3 samples from the stock solution at the specified times, add them (one-by-one) into the 25 cm^3 volumetric flasks in ice–water mixture containing the acidic $FeCl_3$ solutions; homogenize and fill them up with water to level. After 70 minutes, withdraw the 100 cm^3 flask from the thermostat and place it into the icy water. Then, add 2.0 cm^3 0.25 M HCl solution and then 3.0 cm^3 $FeCl_3$ solution in this order[6], homogenize and finally fill up to level by water. Note again that the AcO-SA content of the stock solution at t = 0 s (which equals to the SA content at the end of the reaction) is determined by this latter solution.

The absorbances of the as-obtained 8 solutions are measured at 552 nm wavelength by the photometer; use the same cuvette during the measurements. Occasionally, the spectrophotometer has multiple users during the practice. If this is the case, set the zero absorbance before each measurement using a cuvette filled by water (set $A = 0$ by the *baseline* option). Before the measurements of any solution, the cell should be washed three times by their small amounts (usually 1 – 2 cm^3 is enough if the solution volume is small – for the actual measurement, you need to save at least 3 cm^3 sample!). If there is enough time, the absorbances of the samples can be measured already during the reaction run (see also note 1 at the end of the description). Since the solutions are cold, the outer surface of the cuvette might be covered by condensed fog. This must be wiped off by a (clean!) paper or textile tissue. If the measured absorbance of the sample taken at time t (A_t^{meas}) reaches the range of 1 – 1.2, the solution must be diluted to double volume and the absorbance must be measured again.

For the measurement at higher temperature, the same procedure should be followed. The HCO_3^- / CO_3^{2-} buffer solution prepared at the beginning of the practice shall be used again. However, a new AcO-SA stock solution must be prepared from the remaining part of the pill.

4 Evaluation of the measured data

– Summarize in separate tables the data measured at the two different temperatures and the calculated values needed for the utilization of Equation (3) according to Table 1.

Table 1: Summary of the experimental results.

T_{meas}= .... °C= .... K, A_∞^{meas}= .... , A_∞= ....

Exact time of sampling	t (s)	A_t^{meas}	A_t	$\ln(A_\infty - A_t)$	k (s^{-1})

While filling the tables, remember that the measured absorbance does not equal to A_t in Equation (3). The latter refers to the absorbance, which would be measured if we managed to bring all of the salicylic acid molecules (taken from the reaction mixture) into complexes without dilution. Consequently, A_t can be calculated from the measured values (A_t^{meas}), if dilution is taken into account. Calculate the rate constant point-by-point by Equation (3); determine the average and standard deviation as well (see Appendix).

– Plot (separately) the transformed measurement points on a $\ln(A_\infty - A_t) - t$ diagram at both temperatures! Linear fit should be used to calculate the rate constants (k_{T_1} and k_{T_2}) together with their standard deviation. The parameters of the fitted lines and their error should be determined by the LIN.ILL function of Excel (see Appendix). Compare A_∞ obtained from the fit to that calculated from the measured

[6]The order is important here because if the iron(III) solution is firstly added to the base, a hardly redissolved precipitate is formed.

A_t^{meas} values at both temperatures. To estimate the confidentiality of the measurements, comment on the relative difference (in percentage) of A_∞ obtained on different ways.

- Calculate the half-life of the reaction for both temperatures! How much faster is the reaction at the higher temperature?
- Calculate the activation energy and the pre-exponential factor of the reaction.

Questions

1. Sketch the reaction equation of the aqueous hydrolysis of acetlysalicylic acid using structural formulae!
2. Derive the integrated form of the rate equation of a first-order reaction!
3. How are you going to determine the quantity of the SA formed?
4. Define the Beer–Lambert law!
5. What kind of relationships derived by stoichiometric considerations are valid between the concentrations of AcO-SA and SA during the hydrolysis?
6. How and with which relationship can you determine the rate constant of the pseudo-first order reaction at a given temperature?
7. Define the Arrhenius equation!
8. Derive the formula that provides the activation energy if the pseudo-first order rate constant of the reaction is known at two different temperatures!
9. Briefly (max. 4 – 5 sentences) describe how you are going to prepare the stock solution of AcO-SA (until the step of filing to level)!
10. Note down in 3 – 4 sentences, what problems related to the determination of absorbance can arise and how these problems can be avoided!
11. In a measurement series, we pipetted 2 – 2 cm^3 samples into a (1) 100 cm^3 volumetric flask which contains a base solution, and (2) into a 25 cm^3 flask containing hydrochloric acid and iron(III) chloride solutions. After filling to level, the absorbance of both solutions was measured to be $A_t^{meas} = 0.348$. What are the values of A_t and A_∞ in the measurement series?
12. The rate constant of a first-order reaction is $3.97 \times 10^{-5}\ s^{-1}$. How much time does it take for the formation of the half of the total amount of the product?
13. The rate constants of a first-order chemical reaction are $1.7 \times 10^{-3}\ s^{-1}$ at 33 °C and $5.8 \times 10^{-3}\ s^{-1}$ at 45 °C. What is the activation energy of the reaction?
14. What is the *p*H of the buffer which is prepared by mixing 50–50 cm^3 of 0.7 M NH_3 and 1.2 M NH_4Cl solutions? $K_b = 1.8 \times 10^{-5}$.

Notes:

1. If there is not enough 25 cm^3 volumetric flasks for sampling, then you should prepare as many *quenching* solutions as the number of flasks and, immediately after sampling, the absorbance should be measured. Then, in the liberated flasks, a new *quenching* solution can be prepared.
2. The pre-exponential factor can be expressed as the function of the rate constants and the temperatures in a few ways. The advantage of the first form of Equation (5) is that this can be memorized the most easily, and it demonstrates best that the activation energy needs not to be calculated for the determination of **A**. However, the disadvantage is that the unit of **A** can hardly be *read out* of this expression. However, either from the original form of the Arrhenius equation or from the second form, it is unambiguously seen that the unit of **A** equals to unit of the rate constant. The first form might lead to difficulties in the calculation: for small rate rate constans and high exponents (or a small temperature

change), both the nominator and the denominator can be greater than 1×10^{100}, and thus the regular pocket calculators or spreadsheet softwares may not be able to calculate the result.