## EXERCISE I.11

# DETERMINATION OF THE FREE SALICYLIC ACID CONCENTRATION IN ASPIRIN BY FORMING FE$^{+3}$ COMPLEXES

Maria J. Gil and Víctor Martínez-Merino

*Dto. de Quimica Aplicada, Universidad Pública de Navarra, 31006*

*Pamplona, Spain*

*E-mail: mjg@unavarra.es, merino@unavarra.es*

**INTRODUCTION**

Since its market introduction under the trademark Aspirin® in the year 1899, acetylsalicylic acid has attained a leading position world-wide in the nonprescription therapy of painful, inflammatory, and feverish states. It has proven effective as a general pain reliever, and it is routinely used in a wide range of pain indications, including headache, body and muscle ache, arthritis, and many more.

Today, aspirin is also taken daily by millions of people for its antithrombotic effect. Patients with a prior cardiovascular event, stroke, or evolving myocardial infarction benefit from the life-saving preventative properties of acetylsalicylic acid. The medical community has increasingly recommended routine therapy with low-dose acetylsalicylic acid, which significantly reduces the risk of death from a cardiovascular event. Observational studies have already identified a 40 % reduction in the risk of death from colon cancer among those who frequently use acetylsalicylic acid.
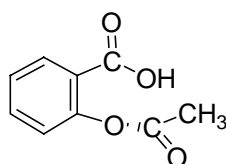


Aspirin (acetylsalicilic acid)

By the late 1800s, salicylates had become the standard drug for the treatment of arthritis. However, the treatment was very harsh and irritating to the stomach. Felix Hoffman, setting out to create a less-irritating medicine, synthesized acetylsalicylic acid (ASA). A deficient method of preparation of aspirin could produce acetylsalicylic acid impurified with free salicylic acid, which presents adverse health effects. A very simple method of detection of the free salicylic acid could be based on the different coordinating capacity of the salicylic derivatives with the octahedral $Fe^{+3}$ cation. Thus, the yellow $FeCl_3$ aqueous solution turns pale pink in contact with an aqueous solution of acetylsalicylic acid, but turns dark blue in the presence of a very light quantity of free salicylic acid.

**SYNTHESIS**

The reaction of formation of the aspirin takes place by treatment of salicylic acid with acetic anhydride, as shown in Scheme 1. A possible method of synthesis could be as follows: In a clean and dry 100-mL Erlenmeyer flask, weigh 6 g of salicylic acid. Carefully add 10 mL of acetic anhydride and 0.2 mL of concentrated sulfuric acid to catalyze the reaction (some heat may be generated). Gently swirl the flask for 10 min; during this time, the solid should dissolve completely. Continue stirring for 30 min to complete the reaction. A white precipitate of aspirin should appear. (If no product has formed, use a glass rod to scratch the inside of the flask, to promote crystal growth). Then, add 50 mL of cold water to the flask. The stirred mixture is then filtered in vacuum with a Büchner funnel, and the solid material washed twice with 25 mL portions of cold water. Subsequently, the solid is dried by passing air through the filter for about 15 min. Then, the white solid is extended on a dry piece of cellulose filter to dry it. When the product is dry, weight it and determine the yield of the reaction.
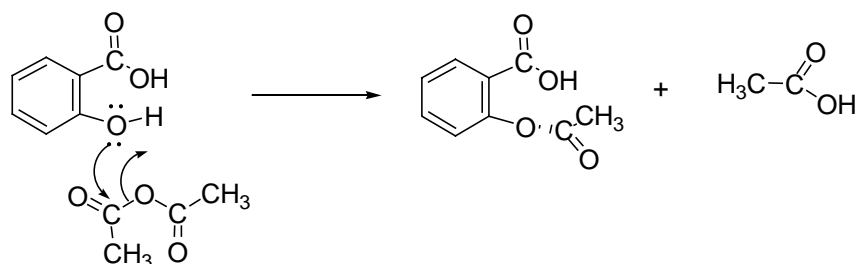


**Scheme 1**

**DETERMINATION OF SALICYLIC RESIDUAL ACID CONCENTRATION BY SPECTROPHOTOMETRY**

The purity of the synthesized aspirin can be tested by addition of $Fe^{+3}$ to a suspension of the product. Phenols (such as salicylic acid) react with ferric chloride to form colored (blue or violet) complexes.

**Color of complex ions [1,2]**

Most *d*-transition ions act as Lewis acids (accept electron pairs) by forming coordinate covalent bonds with ligands (Lewis bases). Complex ions include cationic, anionic, and neutral species ($[Cr(OH_2)_6]^{3+}$, $[Fe(CN)_6]^{3-}$, $[PtCl_2(NH_3)_2]$) and they are colored. The color of complex depends on which metals and ligands are present. Thus, the $[Cr(OH_2)_6]^{3+}$ complex is violet, whereas the corresponding hexamine complex $[Cr(NH_3)_6]^{3+}$ is yellow. However, the $[Co(NH_3)_6]^{3+}$ complex is wine-red as chloride derivative or yellow as nitrate derivative.

A substance appears colored because it absorbs light that corresponds to one or more of the wavelengths in the visible region of the electromagnetic spectrum (4000–7000 A) and transmits or reflects the other wavelengths. A substance absorbs determined wavelengths because the electronic levels inside molecules are quantized so that the absorption of radiation can occur only at values corresponding to the energies required to promote electrons from one level to others.

Ions having a noble-gas electron configuration ($ns^2p^6$, $ns^2p^6d^{10}$, or $ns^2$) do not have electron transitions in the energy range corresponding to visible light; so these ions are colorless in solution (examples are the alkali and the alkaline earth-metal ions, $Zn^{+2}$, $Al^{+3}$, or $Bi^{3+}$).

Most transition-metal compounds are colored, a characteristic that distinguishes them from most compounds of the representative elements. In transition-metal compounds, *d*-orbitals are often split into two sets of orbitals separated by energies $\Delta_{oct}$, as in the case of the octahedral iron complexes, which correspond to wavelengths of the light within the visible region. The absorption of visible light causes electronic transitions between orbitals in these sets. The frequency and, therefore, the wavelength and color of the light absorbed are related to $\Delta_{oct}$. This depends on the crystal field strength of the ligand (L in the figure).
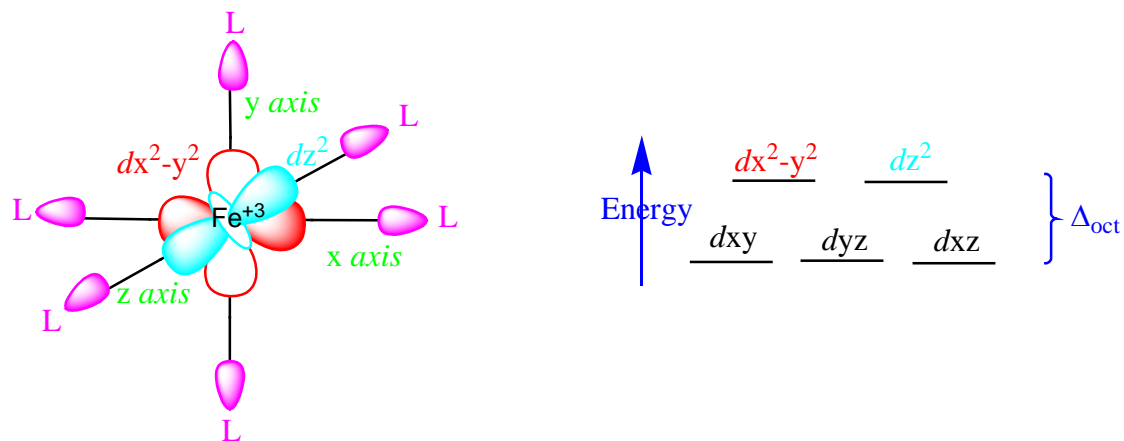
**Fig. 1**

Crystal field theory proposes that the presence of any ion or molecules bound to an ion alters the energy of the 5 $d$-orbitals so that there are energy differences between them.

For example, the approach of six ligands to a metal ion along the $x$, $y$, and $z$ axes, forms a complex with octahedral structure. The orbitals ($dx^2\text{-}y^2$, $dz^2$) have higher energy than the three orbitals ($dxy$, $dxz$, $dyz$) because of the repulsion between filled orbitals from the ligand and the metal (Fig. 1). The energy separation between the two groups of $d$-orbitals is called crystal field splitting $\Delta$ and corresponds to wavelengths of light in the visible region.

Ligands such as $H_2O$ and $F^-$ are weak field ligands and produce small splitting energies $\Delta_{oct}$ (Fig. 1). In this case, the electrons occupy all five non-degenerate orbitals singly before pairing. This is a high spin complex.

On the contrary, ligands such as $NH_3$ or $CN^-$ are strong field ligands and produce large crystal field splitting ($\Delta_{oct}$). The electrons will pair in the lower energy ($dxy$, $dxz$, $dyz$) orbitals before any electrons occupy the higher energy orbitals ($dx^2\text{-}y^2$, $dz^2$). This is a low spin complex.

So transition-metal ions with partially filled $d$-orbitals can absorb photon energy if the energy of the photon approximately matches the difference in energy of the $d$-orbitals. In the process, an electron absorbs the photon energy, going from an energy state to a higher energy half-filled or empty $d$-orbital energy state.

The colors of the complexes are related to $\Delta_{oct}$ and therefore depend on the ligands.

**Determination of salicylic acid (%) in synthesized aspirin by spectrophotometry**

The complex formed between $Fe^{3+}$ ion and salicylic acid is blue, which allows its visual detection. Obviously, the intensity of the observed color is proportional to the complex concentration. Complex concentration can be determined by spectrophotometry, measuring the absorbance of the complex in the visible region (at 540 nm). The Beer–Lambert law states that the proportion of light absorbed by a solute in a transparent solvent is proportional to the number of absorbing molecules in the light path.

$$A = \text{Log}_{10}(I_0/I) \qquad\qquad A = \varepsilon l C \qquad\qquad (1)$$

where:

  $I_0$ = intensity of incident light

  $I$ = intensity of transmitted light

  $\varepsilon$ = molar absorptivity

  $C$ = sample concentration (in mol/L)

  $l$ = cell (path) length (cm); $b = 1$ cm

  $A$ = absorbance

Therefore, the absorbance is in direct relation with the concentration of the absorbent species.

The concentration can be determined by measuring the absorbance of the sample. Several solutions of known molar concentration must be prepared, and their absorbance measured at the desired wavelength. A plot of absorbance versus concentration gives, according to eq. 1, points that can be joined by a straight line. These plots are called **standard curves**.

By addition of $FeCl_3$ to a sample of synthesized aspirin impurified with salicylic acid, a solution of the complex is prepared. Absorbance of the solution is determined. The concentration of the complex, and therefore the purity of the aspirin, will be calculated using eq. 1 (standard curve).

**EXPERIMENTAL PROCEDURE**

**Materials**
- 100-mL Erlenmeyer flask
- Büchner

- 50-mL volumetric flask  (6)
- 1 mL, 2 mL, 5 mL pipettes
- beakers

**Chemicals**

- salicylic acid
- acetic anhydride
- $H_2SO_4$ concentrated
- 5 % $FeCl_3$ (aq)
- 0.2 M salicylic acid (p.m. = 138) (in ethanol)

**Qualitative determination of the residual salicylic acid**

The purity of the synthesized aspirin can be tested by addition of $Fe^{+3}$ to a suspension of the product. Prepare a tube with a small amount (a few crystals) of aspirin. Add water (2 mL) and 1 mL of 0.1 % aqueous solution of $FeCl_3$. The color development is considered a positive test and is an indication of the presence of salicylic acid (try this test on salicylic acid, phenol, benzoic acid, ethanol, and describe the results).

**Quantitative determination of the residual salicylic acid by spectrophotometry**

*Preparation of the calibration curve*

A series of standard complex solutions will be prepared, and their absorbance measured at 540 nm in a spectrophotometer. Concentrations of solutions must be ranged between $1 \times 10^{-4}$ and $8 \times 10^{-4}$ M.

- Prepare five 50-mL volumetric flasks (1–5). In each one, put the volume (mL) of solutions indicated in the table below. Add water up to the mark.

|  | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| Salicylic acid (0.01 M) | 0.5 mL | 1 mL | 2 mL | 3 mL | 4 mL |
| $FeCl_3$ (aq) | 0.1 mL | 0.2 mL | 0.4 mL | 0.6 mL | 0.8 mL |
| **Dilute to 50 mL exactly with distillated water** | | | | | |
| **C (mol/L)** <br> Salicylic acid concentration | $1 \times 10^{-4}$ | $2 \times 10^{-4}$ | $4 \times 10^{-4}$ | $6 \times 10^{-4}$ | $8 \times 10^{-4}$ |
| **Absorbance** (540 nm) | | | | | |

- Add distillated water to fill the flask to 50 mL.

- Swirl the flash to obtain homogeneous solutions.

- Measure the absorbance of the solutions (wavelength = 540 nm).

- Plot the calibration curve. absorbance vs. concentration (M).

*Preparation of the sample*

Place 0.2 g of aspirin into a 50-mL flask. Add ethanol (5 mL approximately) to dissolve it. A solution of $FeCl_3$ (1 mL) is added, and the flask is filled with it up to the mark (if a precipitate appears, add a little more ethanol and dilute after that to 50 mL). The observed color must be included into patron solutions scale. If the observed color is too intense, dilute the solution.

*Determination of salicylic acid concentration in aspirin*

Measure the absorbance of the sample solution by means of the spectrophotometer. Use the calibration curve to calculate the concentration of the complexed salicylic acid (C) in the solution.

*% Salicylic acid in aspirin*

$$\text{salicylic acid ( \% )} = \frac{C \times 50 \times 10^{-3} \times 138}{0,2} \times 100$$

If the percentage of salicylic acid is >10 %, aspirin could be recrystallized from toluene. Recrystallize 0.5 g of just-synthesized aspirin. Repeat the test of the ferric chloride. Compare the color differences.

**REFERENCES**

1. K. W. Whitten, R. E. Davis, M. L. Peck and G. G. Stanley. *General Chemistry*, 7th ed., Thompson Learning, London (2004).

2. R. H. Petrucci, W. S. Harwood, F. G. Herring. *Química General*, 8th ed., Prentice Hall, Madrid (2003).

**Víctor Martínez-Merino**

*merino@unavarra.es*

High standards in safety measures should be maintained in all work carried out in Medicinal Chemistry Laboratories. The handling of electrical instruments, heating elements, glass materials, dissolvents and other inflammable materials does not present a problem if the supervisor's instructions are carefully followed.

This document has been supervised by Prof. Victor Martinez-Merino (merino@unavarra.es) who has informed that no special risk (regarding toxicity, inflammability, explosions), outside of the standard risks pertaining to a Medicinal Chemistry laboratory exist when performing this exercise.

If your exercise involves any "special" risks, please inform the editor.