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Guide for contributors to the Australian Journal of Education in Chemistry

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Aspirin and Its Colored Complexes: How This Drug Reacts with Metal Ions

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Abstract

A laboratory experiment for extraction and purification of acetylsalicylic acid from commercial aspirins and its reaction with Fe(III) and Cu(II) solutions. The complexation reactions are easily observed thanks to the intense color variations, nevertheless the identity of the extracted and synthesized compound has been verified using thin-layer chromatography, UV-vis, fluorescence and infra-red spectroscopy, and mass spectrometry. This experiment demonstrates that varying the ligand and the transition metal can give rise to different structures, and hence different optic properties.

Keywords: First-Year Undergraduate, Second-Year Undergraduate, Demonstrations, Interdisciplinary / Multidisciplinary, Inorganic Chemistry, Hands-On Learning / Manipulatives, Crystal Field / Ligand Field Theory, Dyes / Pigments, Organometallics.

Introduction

General undergraduate laboratory experiments should to reflect modern research practice and it is essential that they include a range of elements, and that synthetic tasks are accompanied by qualitative analysis and complete characterization.

The first purpose of this experiment is to introduce undergraduate students in a chemistry laboratory to the extraction and purification of acetylsalicylic acid from commercial aspirins and its showy reaction with Fe(III) and Cu(II) solutions.

In this paper we report a sequence of experiments planned for the general chemistry laboratory based on a familiar substance: aspirin. Aspirin (acetylsalicylic acid, **Asa**) is a drug compound familiar to virtually all students. Even if labs involving aspirin are common, they are useful because these experiments aid general chemistry students relate chemistry to real-world problems.

Indeed, the second purpose of the experiment is to employ the extracted and synthesized complexes to verify the identity of the molecular structure using thin-layer chromatography, UV-vis, infra-red spectroscopy, and mass spectrometry.¹⁻³

The use of aspirin makes the experiment close to everyday life experience. In the first experimental part, the strong color of the complex products is easily observable and has a strong emotional and cognitive impact on students.^{4,5}

The laboratory experiment is designed for general chemistry laboratory course, with a prerequisite chemistry course, to reinforce how to use techniques together, which is a necessary skill in organic and inorganic chemistry. The objective is to provide students with an experience in two major areas common to organic chemistry: (1) extraction and purification of pharmaceutical compounds, and (2) synthesis of metal ions complexes and their photophysical and structural characterization, comparison between ligand and corresponding complex photophysical behavior.⁶⁻⁸

Herein, we investigate the extraction of the acetylsalicylic acid (Aspirin) from aspirin commercial tablet and the formation of several Fe(III) and Cu(II) complexes. One of the goals of this demonstration is to show the formation of complexes with aspirin as a ligand with Fe(III) and Cu(II) as the ions to be coordinated.⁹ The experiment uses two non-colored but fluorescent products to synthesize the corresponding deeply colorful and non-fluorescent complexes, this allows students to apply the concepts of the Crystal Field Theory and observe the modification of the spectral and optical properties after the complexes formation. Furthermore, metal coordination complexes of biological molecules have much potential for designing novel therapeutic and diagnostic agents, which target specific properties and show reduced side effects, avoidance of resistance and improved selectivity, as they can be used for treating a wide range of important human diseases.¹⁰

Additional challenges of this experience are the possibility to better understand the mechanism of action of small molecules (such as aspirin), in order to make a further evaluation and modulation of the chemical composition and reactivity. Of particular importance in the field of synthetic and biological chemistry are Fe(III) and Cu(II) complexes, because of the role these elements plays in biological systems.¹¹⁻¹³ The interaction of transition metal ions with drugs is a subject of considerable interest, as aspirin has been previously reported to form molecular complexes with Ag(I), Zn(II), Cu(II) and Co(II) ions.¹⁴

Experimental Overview

The timespan of this experiment is two 4 h sessions, with a pre-lab quiz administered at the beginning of each session (See SI: Pre-work quizzes and Post lab questions). The experiment is ideally carried out in groups of 3–4 students.

Acetylsalicylic acid extraction from commercial aspirins.

In the first session, students extract and purify acetylsalicylic acid from commercial aspirins tablet.

Commercial aspirin contain a list of ingredients such as starch, lactose monohydrate, purified talc, silica dioxide, sodium carbonate, zinc stearate, carnauba wax, hypromellose or others depending upon the pharmaceutical recipes.¹⁵

The aspirin collected has been purified by recrystallization. In this purification method, the crude aspirin (2/3 commercial tablet) will be dissolved in a small amount of warm ethanol or acetone and filtered. Water will then be added at the filtrate and the solution will be cooled slowly and then chilled. The acetylsalicylic acid recrystallize, and the solid impurities (excipients) should remain dissolved in the solution (See SI: Instructor lab manual notes for experiment). The solid aspirin will again be recrystallized two times and tested for purity. The yield, appearance of the ligand, and melting point are recorded.

Qualitative Analysis

TLC is performed on TLC sheets coated with 0.25 mm layers of silica gel 60 F₂₅₄. After the application of the extract (about 10 μ l), the sheets are developed in paper-lined all-glass chambers with solven, previously left to equilibrate for at least 10 min.

Spots are visualized with a UV lamp. The distance to the center of the spot (d_{spot}) and the solvent (dichloromethane-ethanol 94-6) front distance (d_{solv}) from the spot line are measured with a ruler, and the Rf (retention factor) for the spot is calculated from $R_f = d_{\text{spot}}/d_{\text{solv}}$. The TLC method uses commonly available supplies and laboratory glassware, facilitating its use in the general chemistry laboratory, such as 100 ml beakers, filter paper, and aluminum foil to cover the beaker (the "TLC chamber"). The TLC developing solvent efficiently separates acetylsalicylic acid ($R_f = 0.53$ in dichloromethane-ethanol 94-6).

Using a melting point apparatus, students determined the melting point of their crude and recrystallized aspirin samples, recording the value as a range from when melting commenced to when all the sample was liquid (In order to get a meaningful result for the melting point determination, the solids must be dry). The melting point of pure aspirin is 135°C, and the melting point of salicylic acid is 158°C. Four students (of 20) reported melting points were lower than literature values, indicating impurities. The students were asked to comment on the purity of their extracted aspirin based on its melting point.

Synthesis Of complexes

In the first session, each group uses the previously extracted acetylsalicylic acid to synthesize the corresponding Fe(III) and Cu(II) complexes (see fig. 1, 2).

The collected aspirin has been employed as a solution (2.5ml, 10⁻²M) for reaction with acid metal ions solutions (Fe(III), Co(II), Ni(II), Cu(II), Zn(II)) 2.5ml of 10⁻²M. Only Fe(III) ion reacts to form a purple complex. Salicylic acid (**Sal**) contains a phenol group, but acetylsalicylic acid does not. Therefore, if you add FeCl₃ to an aspirin sample and you see a purple color, it

means that there is some salicylic acid present. Co(II), Ni(II), Cu(II) and Zn(II) ions don't show any change in color(see fig. 1).

Acetylsalicylic acid tends to hydrolyze in solution yielding salicylic acid, and we experimentally observe that the Fe complex did not originate from the **Asa** but from the salicylic acid present as an impurity, or as a hydrolysis product of the **Asa**.

Edwards first showed that the rate of hydrolysis of aspirin is independent of pH between pH 4 and 8. The hydrolysis reaction has been the subject of a number of studies, particularly by Garrett.^{16,17} Edwards considered that hydrolysis in the pH-independent region involves attack by a molecule of water on the aspirin anion.¹⁸ Typically esters are subject to hydrolysis by both aqueous acids and bases and **Asa** is an ester. Preparations having elevated amount of aspirin often smell like acetic acid because aspirin decompose through hydrolysis in wet conditions, yielding salicylic and acetic acids. **Asa** is stable in dry air, but gradually react in contact with moisture, otherwise the hydrolysis proceeds more speedily in solution with acid or alkalis.

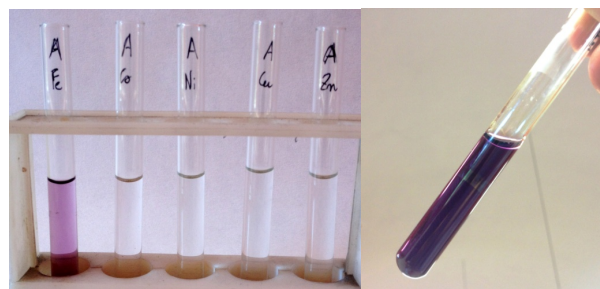


Figure 1. a) From left to right: test tubes filled with 2.5ml of 10⁻²M metal ion water solutions (Fe(III), Co(II), Ni(II), Cu(II), Zn(II)) and 2.5ml of 10⁻²M acetylsalicylic acid; observe the intense purple color in the Fe tube due to the formation of the Fe(III) complex.

Iron Salicylate

In neutral or acid solution, Fe(III) ion is present as the $[\text{Fe}(\text{H}_2\text{O})_6]^{+3}$ ion. This ion can bind to salicylic acid because water is replaceable in iron complexes. One water can be replaced by the negative carboxylic acid oxygen ion in salicylic acid as salicylate. The phenolic oxygen of salicylic acid can also replace one of the waters in the $[\text{Fe}(\text{H}_2\text{O})_6]^{+3}$ complex. The iron salicylate complex has an intense violet color due to the ligand-to-metal charge transfer transition, which moves the absorption maximum from the ultraviolet to the visible region. In aspirin, the -OH group of salicylic acid has been replaced by an ester group which prevents the formation of a second bond by the phenolic oxygen. The resulting complex with aspirin shows only a slight yellow color, not very different from that of $[\text{Fe}(\text{H}_2\text{O})_6]^{+3}$ itself.

Copper Aspirinate

Acetylsalicylic acid was dissolved in aqueous NaHCO₃. Sodium hydroxide is not suitable, because it will completely hydrolyze acetylsalicylic acid into salicylic acid and sodium acetate. An excess of acetylsalicylic acid is used to avoid the possibility of unreacted carbonate anions precipitating the copper as copper carbonate. The resulting solution is filtered to remove

any undissolved compound and is added to a solution of Cu(II) cations (Cu(II) nitrate, acetate or sulfate are suitable) (See SI). Bright blue crystals of copper aspirinate immediately form and precipitate (see fig. 2). The crystals can then be filtered from solution, washed, and dried.¹⁹

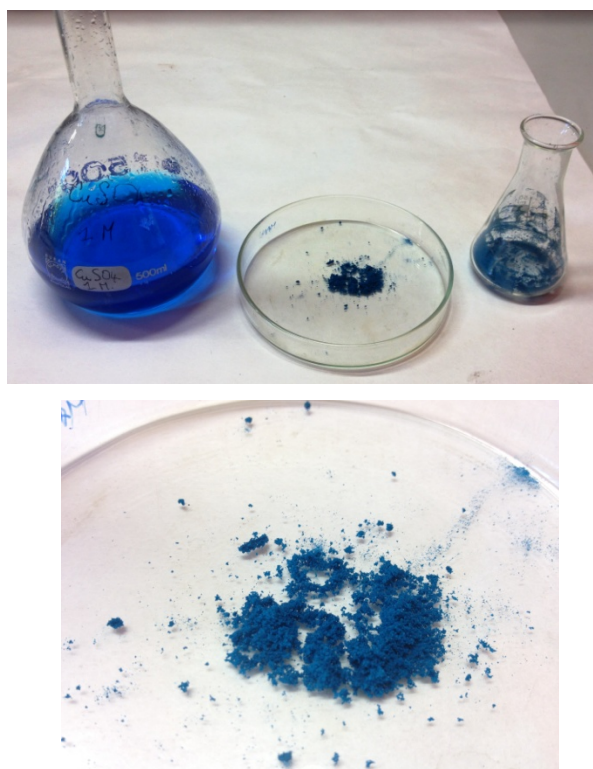


Figure 2. a) (top) Left to right: copper sulfate solution (1M), copper aspirinate precipitate from synthesis. b) (bottom) The same copper aspirinate precipitate, observe the intense deep blue-green color.

The reaction is very simple and can be carried out without special precautions related to oxygen or water presence. Even in the presence of impurities in the acetylsalicylic acid, the synthesis leads to the desired deep blue colored product because of the high selectivity of the reaction between metal and ligand. In any examined case we always observed an intense color changing due to the complex product.

In the second session, students record the full IR, ESI-MS, UV-visible absorption and emission spectra for solutions of the ligand, metal salt and metal complex. The wavelength of maximum absorbance/emission, intensity at this wavelength, and solution color are recorded for each solution.

Recrystallization and filtration, column chromatography and TLC are frequently taught as part of a second-year undergraduate organic chemistry laboratory curriculum in many institutions. However, these techniques are often taught in separate laboratory periods, and students sometimes have difficulty grasping the importance of when and how these techniques can be used in combination with IR, UV-vis, ESI-MS and NMR to enable structural determination of unknown compounds.

Result And Discussion

This experiment has been performed in an intermediate general chemistry laboratory course with 20 enrolled students. Students in this course had completed first-year general chemistry, comprising introductory organic, inorganic, physical and theoretical chemistries.

As previously stated, this experiment is split up into two separate sessions: the first being the extraction and purification of Aspirin and synthesis of corresponding metal complex; the second being a UV-visible, fluorescence, infra-red, mass spectroscopic characterization exercise, consisting of obtaining the complete spectra for comparison with literature data, as well as establishing the metal:aspirin stoichiometry.

All students reported the appearance of Aspirin as off-white crystals, with 14 (of the 20 students) collecting crystals following recrystallization, and the remaining 6 obtaining a white powder, even after purification, likely due to extremely rapid cooling during recrystallization. Yields ranged from 22 to 66%, with an average of 44%.

All students easily appreciate the strong color variations, facilitating the understanding of the Crystal field theory and discussion of the modification in the energy of the *d*-orbitals during the complex formation.

Spectroscopic Characterization

The acetylsalicylic acid is well known in literature for its photophysical properties.^{20,21} Two absorption shoulders fall in the wavelength range from 300 nm to 380 nm in water solution, while almost no absorption beyond 400 nm are observed/detected. The acetylsalicylic acid show large Stokes' shifts (nm), high quantum yield (under UV excitation) and an intense fluorescent emission ($\lambda_{exc}=335\text{nm}$) at wavelengths in the 350-450 nm range in water (see fig. 3).²²

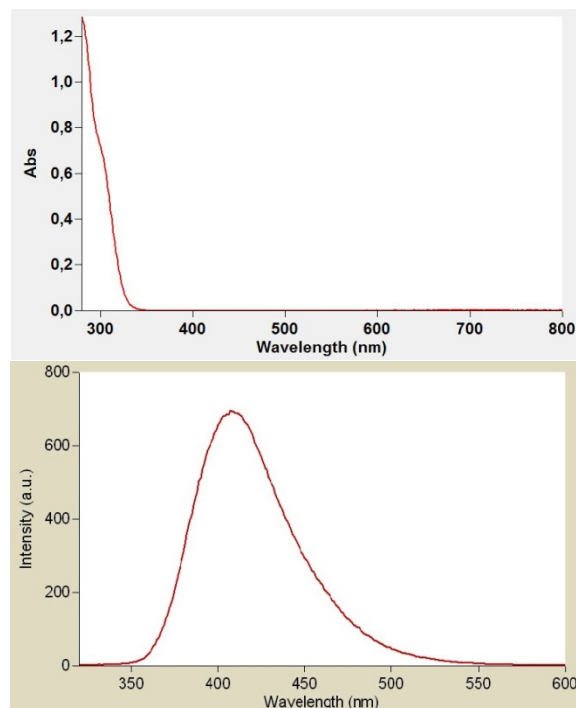


Figure 3. UV-Visible absorption spectra (top) and emission spectra (bottom) of acetylsalicylic acid, recorded in water solution.

Absorption and emission spectra dilute solutions ($\sim 10^{-4}$ M) of Fe(III) (water) and Cu(II) (water and DMSO, 50%) complexes are presented in Figure 4 and 5, and their absorption wavelengths are reported in Table 1. A strong color changing in the products solutions is easily observable because of the high molar absorption coefficient as previously reported. On the contrary, the complexes show no emission, it is evident that the optical characteristics of the complexes may be very different from those of the ligands (See Table 1).

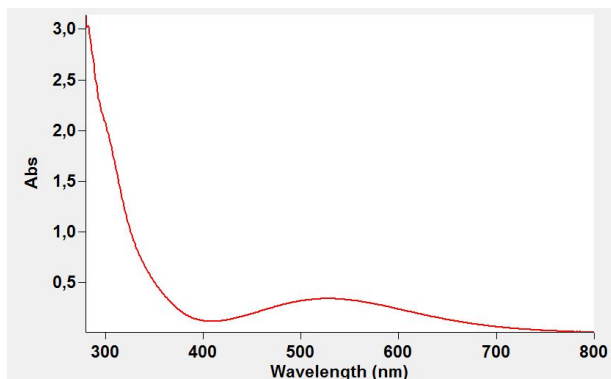


Figure 4. UV-Visible absorption spectra of Fe-acetylsalicylic acid complex, recorded in water solution.

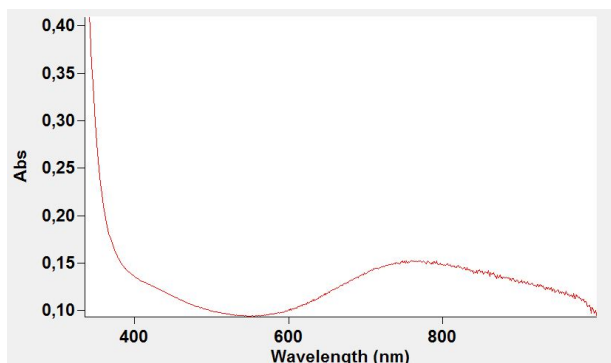


Figure 5. UV-Visible absorption spectra of Cu-acetylsalicylic acid complex recorded in DMSO-water solution (1:1).

Table 1.

| Photophysical properties of the acetylsalicylic acid and corresponding complexes (* for shoulder). | | | |
|----------------------------------------------------------------------------------------------------|----------------------|---------------------|--------------------|
| Compound | λ_{abs} (nm) | λ_{em} (nm) | Stokes' shift (nm) |
| acetylsalicylic acid | 285*, 300* | 408 | 108 |
| salicylic acid | 237, 303 | 450 | 147 |
| Fe complex | 530 | x | x |
| Cu complex | 760 | x | x |

Most *d*-transition metal ions act as Lewis acids (accept electron pairs) by forming complex with covalent bonds with ligands (Lewis bases). Complex include cationic, anionic, and neutral species ($[\text{CoCl}_2(\text{NH}_3)_4]^+$, $[\text{Mo}(\text{CN})_8]^{4-}$, $[\text{Cr}(\text{H}_2\text{O})_6]\text{Cl}_3$) and they are colored. The color of complex depends from the metals and the ligands type. Most transition-metal compounds are colored, a characteristic that distinguishes them from most compounds of the representative elements. In

transition-metal complexes, *d*-orbitals are frequently split into two sets energy levels separated by definite energies. The absorption of visible light causes electronic transitions between orbitals in these energy levels. The wavelength and color of the light absorbed are related to structure and nature of the complex because are connected to the energy of the *d*-orbitals. Crystal field theory proposes that the presence of any ligand bound to a metal ion modify the energy of the 5 *d*-orbitals so that there are energy modifications between them. So transition-metal ions with partially filled *d*-orbitals can absorb photon energy if the energy of the photon approximately matches the difference in the *d*-orbitals. In this phenomena, an electron absorbs the light energy, going from an energy level to a higher energy half-filled or empty *d*-orbital energy orbital. The colors of the complexes strongly depend on the ligands and the metal nature.

The IR spectra show that acetylsalicylic acid behaves in a bidentate manner, coordinating via the two oxygen atoms of the acidic group with the displacement of a hydrogen atom (see fig. 6). This mode of chelation is supported by the disappearance of the peak at 1680cm^{-1} and $2500\text{--}2920\text{cm}^{-1}$ (g carboxylic acid) and other peaks resulted shifted to lower frequency, indicating participation in coordination (see Table 2).²³

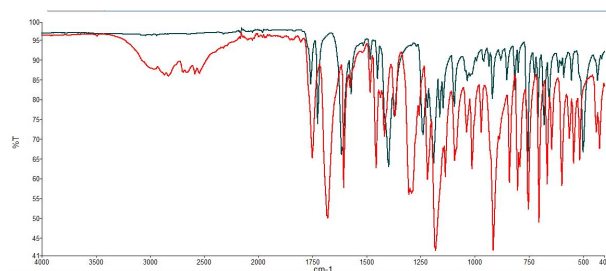


Figure 6. IR spectra of Extracted acetylsalicylic acid (red line) and copper-aspirinate dried at 85°C for 30 min (green line).

Table 2.

| Compound/signal (cm^{-1}) | Band Assignment |
|--------------------------------------|----------------------------------|
| Aspirin sample | |
| 2500- 2920 | γ (O-H) carboxylic acid |
| 1751 | γ (C=O) acetylcarbonyl |
| 1680 | γ (COOH) carboxylic acid |
| 1607 | γ (C=C) ring |
| 1455 | γ (C=C) ring |
| 1178 | γ (C-O) |
| Cu-Aspirinate | |
| 1760 | γ (C=O) free acetyl group |
| 1730 | γ (C=O) binded acetyl |
| 1607 | γ (C=C) ring |
| 1455 | γ (C=C) ring |

Mass-Spectrometry Characterization

The positive mass spectrum reported in Figure 7 is relative to the Fe solution with acetylsalicylic acid extracted. In this case is the salicylic acid that forms a Fe^{3+} complex (salicylic acid (**Sal**), $\text{C}_7\text{H}_6\text{O}_3$, $\text{MW}=138,12$, salicylate, $\text{C}_7\text{H}_5\text{O}_3$, $\text{MW}=137,02$). Figure

7 shows two major peak at $m/z=330.06$, attributable to the corresponding complexes $[\text{Fe}(\text{Sal})_2]^+$ $m/z=330.09$ (see fig. 7). The isotopic patterns confirm peaks assignment (free mass spectra simulators are available online to simulate $\text{FeC}_{14}\text{H}_{10}\text{O}_6$ peak $\text{MW}=330.09$, see fig. 8).²⁴

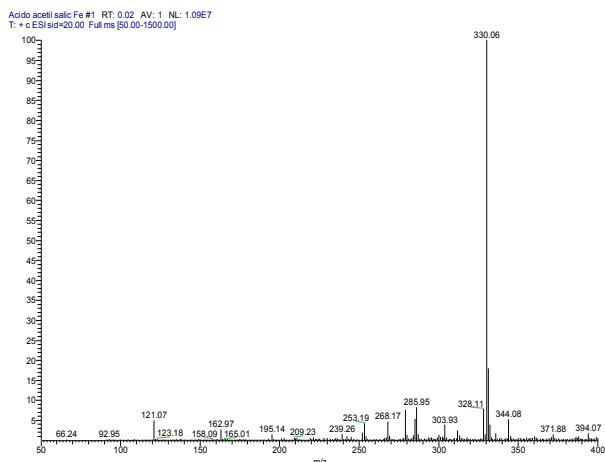


Figure 7. ESI-MS spectrum of Fe ($[\text{Fe}(\text{Sal})_2]^+$ $m/z=330.09$).

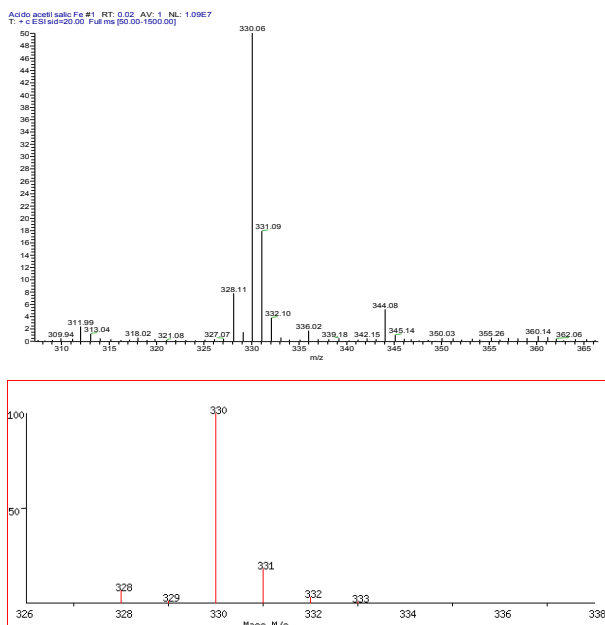


Figure 8 Top) ESI-MS spectrum of Fe-acetylsalicylic acid complex; Bottom) simulate ESI-MS spectra of peak $\text{FeC}_{14}\text{H}_{10}\text{O}_6$ peak $\text{MW}=330.09$.

The negative mass spectrum reported in Figure 9 is relative to the Cu(II) solution with acetylsalicylic acid extracted. In this case is the acetylsalicylic acid that forms a copper complex (acetylsalicylic acid (*Asa*), $\text{C}_9\text{H}_8\text{O}_4$ $\text{MW}=180,16$). Figure 9 shows a major peak at $m/z=600.03$, attributable to the corresponding complexes $[\text{Cu}(\text{Asa})_3]^-$ $m/z=600.04$ (*Asa* as acetylsalicylate, $\text{C}_9\text{H}_7\text{O}_4$ $\text{MW}=179,16$), (see fig. 10 top).

The isotopic patterns confirm peaks assignment (free mass spectra simulators are available online to simulate $\text{CuC}_{27}\text{H}_{21}\text{O}_{12}$ $\text{MW}=600.99$), (see fig. 10 bottom).

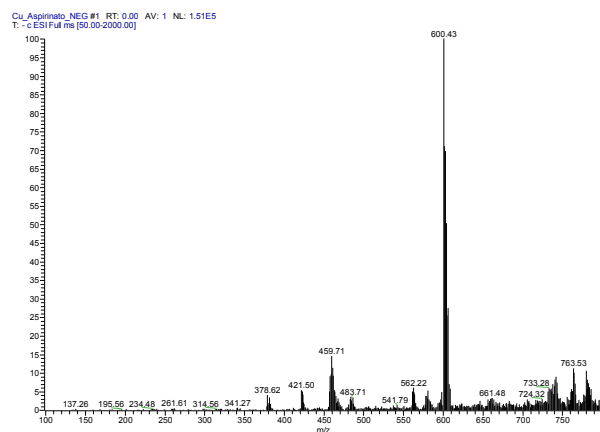


Figure 9. ESI-MS spectrum of Cu complex product.

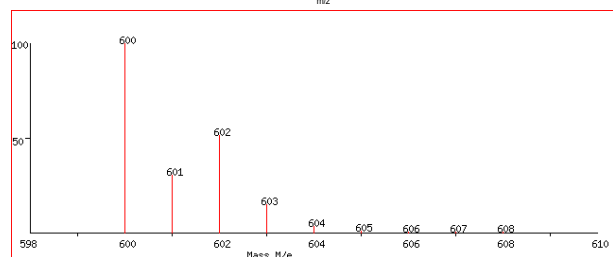
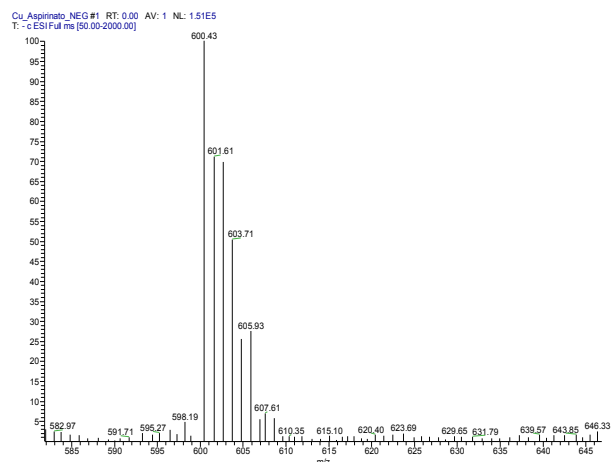


Figure 10. Top) ESI-MS spectrum of Cu-acetylsalicylic acid solution; Bottom) simulate ESI-MS spectra of peak $\text{CuC}_{27}\text{H}_{21}\text{O}_{12}$ peak $\text{MW}=600.99$.

The clear fragmentation obtained in MS/MS spectrum revealed that the complex can lose a ligand ($600.43-180.16=420.27$; see peak $m/z=420.76$), see figure 11. The stoichiometry of the two complexes are confirmed by isotopic pattern (see free mass spectra simulator online).

Solid copper aspirinate is a neutral dimer $\text{C}_{36}\text{H}_{28}\text{Cu}_2\text{O}_{16}$, (dicopper 2-acetyloxybenzoate) but in DMSO/water solution the $[\text{Cu}(\text{Asa})_3]^-$ $m/z=600.04$ is present as demonstrate from the ESI-MS spectra.

Hazards

Students must wear safety glasses and laboratory coats at all times. Extraction and recrystallization, synthesis and TLC analysis should be performed in a fume hood. The Cu(II) and Fe(III) salts are harmful if swallowed and by inhalation.

Ethanol is highly flammable. Students are provided with access to the material safety data sheets for all chemicals, and are asked questions on safety aspects of

the experiment as part of their prelab work. Remember to wear safety glasses when preparing or presenting the experiment. All starting materials waste can be disposed in non-hazardous waste (See SI: Work instructions for service room).

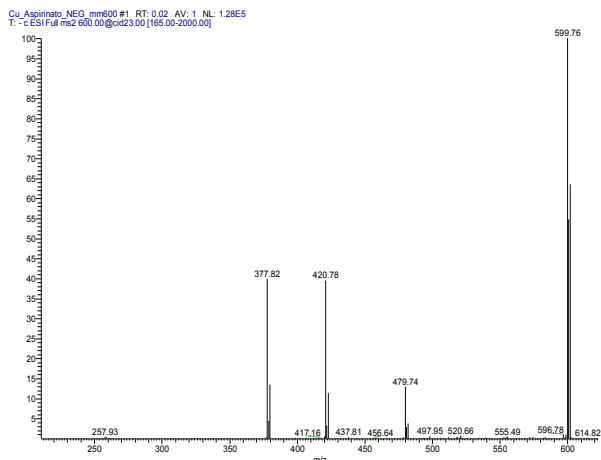


Figure 11. ESI-MS-MS spectrum of Cu complex product, peak $m/z=600$.

Conclusions

This laboratory experiment is appropriate for an intermediate and general course. It involves simple, inexpensive starting materials, and demonstrates a number of important principles related to modern chemistry research.

The experiment has the following key learning outcomes:

- Improve the synthetic skills (extraction-recrystallization of acetylsalicylic acid), assessed by yield and appearance of the extracts.
- Exposure to a range of analytical techniques (TLC, Mass spectrometry, absorption and emission spectroscopy, Infra-Red spectroscopy), assessed by reported spectra.
- Improve data analysis (comparison between experimental and reported spectra).
- Improve understanding (for example, of the relationship between absorption spectra and structure of ligand and complexes), assessed through planning of the experimental report.

Students are able to observe that the identity structure of the acetylsalicylic acid and the employed metal ion affected the photophysical behavior of the product itself. Furthermore, students are required to compare measured spectra to literature values, which let them experience literature search.

In conclusion, in this experience it is possible to observe an example of purification of organic compounds from common drug and the formation of two interesting metal complex with strong color changing.

At a somewhat more advanced level, other things which can be included in the discussion are the structures of pharmaceutical products as well as the corresponding metal complex derivatives, and their optical and structural behavior, as students can properly understand the results.²⁵

The paper is focused on Fe(III) and Cu(II), owing to their strong color changes of the products, anyway the experiment could readily be expanded to include other metal ions as previously reported.

These compounds represent an interesting class of colored complexes with potential interest for pharmaceutical applications. Moreover, high reaction yields, absence of catalysts, high accessibility and stability, ease of handling and preparation make this procedure useful for student laboratory.

Furthermore, students were required to compare measured ESI-MS/MS, UV-vis, IR spectra to literature values, which gave them the experience of searching the literature for such information.

Crystal Field Theory can be used to justify the diverse colors of the Fe(III) and Cu(II) solutions and of the different complexes or, conversely, the colors can be used to give a visual demonstration of the theory.

Summary

This laboratory experiment was appropriate for an intermediate, general or inorganic chemistry course. The experiment used not toxic, simple, inexpensive starting materials, and demonstrated a number of important principles related to modern chemistry research such as Crystal Field Theory, the interaction of transition metal ions with drugs, and improve the synthetic skills of the students, and also exposes them to a wide range analytical techniques.

Supporting Information

CAS numbers, Instructor lab manual notes for experiment, Pre-work quizzes, Post lab questions, Student lab manual notes for experiment, Work instructions for service room.

Acknowledgments

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Supplementary Information: Aspirin and Its Colored Complexes: How This Drug Reacts with Metal Ions

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CAS NumbersCopper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) 10031-43-3

R-phrases R22 R36/38 R50/53 R8

S-phrases S26 S37/39 S60 S61

Iron(III) chloride (FeCl_3) 7705-08-0

R-phrases R34

S-phrases S26 S37/39

Ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) 64-17-5

R-phrases R 11

Dichloromethane (CH_2Cl_2) 75-09-2

R-phrases R36/37/38, R40, R67

S-phrases S23, S24/25, S36/37

Sodium bicarbonate (NaHCO_3) 144-55-8

Hydrochloric acid (HCl 1M) 7647-01-0

R-phrases R34, R37

S-phrases (S1/2), S26, S45

NOTES FOR THE INSTRUCTOR

Here we present some of our procedures and results to facilitate the work of the instructor.

1. Reagents, standard solutions and materials

Copper nitrate $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (or Copper Sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)), ethanol, dichloromethane, HCl, FeCl_3 , NaHCO_3 all of technical grade, were used as received from commercial suppliers without further purification; in addition, deionized water from a Milli-Q system (Millipore) was used. TLC has been performed on 10×20 cm TLC sheets, coated with 0.25 mm layers of silica gel 60 F₂₅₄ (E. Merck). It is possible to use different aspirin commercial tablet.

2. Disposal of wastes and unused solutions

All starting materials (aspirin) waste can be disposed in non-hazardous waste. All solutions (metal solution, solvent, complexes) in waste container in hood. Waste must be disposed of in accordance with federal, state and local environmental control regulations. The organic solvents used in this experiment are harmful for human beings and the environment and all experiments with them should be done under fume hood. After usage, the wastes should be disposed in the proper containers, taking special attention to chlorinated hydrocarbons.

3. Spectroscopic characterization

UV-Vis spectra were recorded on a CARY-60 spectrometer, fluorescence measurements were recorded using Cary Eclipse Varian V. The range for fluorescence emission recording was between 300 nm and 700 nm, $\lambda_{\text{exc}}=335\text{nm}$, water solutions.

The FT-IR spectrum of solid Cu-Aspirinate product was recorded in the region $400\text{--}4000\text{ cm}^{-1}$ on Spectrum Two (Perkin Elmer) spectrophotometer in ATR mode (number of scan=16).

ESI (Electro Spray Ionization) experiments were conducted with a Thermo Finnigan Advantage Max Ion trap spectrometer in positive ion acquiring mode; sheath gas flow rate was set at 25 (arbitrary unit), auxiliary gas flow rate at 5 (arbitrary unit), spray voltage at 3.25 (KV), capillary temperature at 270 C, capillary voltage at 7 (V), and tube lens offset at 60.00 (V). Nitrogen was used as sheath and auxiliary gases.

4. Direct experience facilitating discussion with students

The students in the Faculty of Chemistry were surprised seeing the intense color variation of the metal ion solutions reagent. Previously, they were advised that give gives a red coloration with iron and blue precipitation with Copper. Therefore, this experience leads to some considerations concerning the stability and the kinetics of complexes, which are linked to the crystal field theory, spectrochemical series and the energy separation between the *d*-orbitals in the metal ion complexes.

PRE-WORK QUIZZES

- 1). According to the MSDS, what risk phrase applies to ethanol?
 - a. R5 – Heating may cause an explosion
 - b. R11 – Highly flammable
 - c. R36 – Irritating to eyes
 - d. All of the above
- 2). At the end of the first step of this experiment (extraction of aspirin), you collect your product by filtration. Where will you dispose of the waste filtrate?
 - a. In the organic waste
 - b. In the halogenated organic waste
 - c. Down the sink
 - d. In the rubbish bin
- 3). According to the MSDS, what route of exposure of copper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) has the most severe (toxic) effects?
 - a. Eye exposure
 - b. Skin exposure
 - c. Ingestion
 - d. Inhalation

POST LAB QUESTIONS

- 1) How to verify the aspirin presence in the extract?

Using thin-layer chromatography and the corresponding product purity by melting point apparatus.

- 2) How do you explain the color change from yellow to purple in the Fe(III) solution after aspirin addition?

In neutral or acid solution, Fe^{3+} ion is present as the $[\text{Fe}(\text{H}_2\text{O})_6]^{+3}$ ion. This ion can bind to salicylic acid because water is replaceable in iron complexes. One water can be replaced by the negative oxygen ion in salicylic acid as salicylate. In the extracted aspirin the acetylsalicylic acid tends to hydrolyze in solution yielding salicylic acid, and we experimentally observe that the Fe complex did not originate from the aspirin but from the salicylic acid present as an impurity, or as a hydrolysis product of the acetylsalicylic acid.

- 3) How do you explain the precipitation after the addition of Cu(II) solution to the aspirin solution?

In neutral or acid solution, Cu^{2+} ion is present as the $[\text{Cu}(\text{H}_2\text{O})_6]^{+2}$ ion. This ion can bind to acetylsalicylic acid because water is replaceable in copper complexes. One water can be replaced by the carboxylic group in acetylsalicylic acid to form final copper aspirinate complex.

- 4) In this experiment, you wash your products with solvent: ethanol. Why do you need this washing step, and what temperature solvent should you use?

The washing step will help remove any impurities. The solvent must be kept ice-cold to ensure that only a minimal amount of product is dissolved into the filtrate.

- 5) Why is IR spectroscopy used to identify molecules?

IR spectroscopy is used to identify molecules because many times, each functional group possesses a characteristic stretching frequency that can be quickly identified. After complexation the characteristic peaks show a typical shift because change the stretching frequency.

- 6) What band stretching frequency would you expect to see for each of the three molecules isolated in this lab? Which functional group do these correspond to?

For the aspirin molecule and relative complexes: Peaks corresponding to the CH bonds should be found between 2800 and 3200 cm^{-1} (both sp^2 and sp^3 hybridized C-H stretching frequencies). Finally, peaks corresponding to the C=C bonds should be present between 1500 and 1650 cm^{-1} . In addition, a peak should be observed between 1700 and 1800 cm^{-1} indicating a C=O bond.

- 7) Why is ESI-MS spectrometry used to identify molecules?

Mass spectrometry is an analytical technique that can provide both qualitative (structure) and quantitative (molecular mass or concentration) information on analyte molecules after their conversion to ions. The ions then travel through the mass analyser and arrive at different parts of the detector according to their mass/charge (m/z) ratio. ESI-MS spectrometry gives important information about the charge, the mass, fragmentation and therefore the stoichiometry of the complex and the isotopic distribution also confirms the type and stoichiometry of metal ions.

- 8) Why both salicylic acid and acetylsalicylic acid show no color and the corresponding Fe and Cu complexes appear deeply colored?

In transition-metal complexes, *d*-orbitals are frequently split into two sets energy levels separated by definite energies. The absorption of visible light causes electronic transitions between orbitals in these energy levels. The wavelength and color of the light absorbed are related to structure and nature of the complex because they are connected to the energy of the *d*-orbitals. Crystal field theory proposes that the presence of any ligand bound to a metal ion modifies the energy of the *d*-orbitals so that there are energy modifications between them. So transition-metal ions with partially filled *d*-orbitals can absorb photon energy if the energy of the photon approximately matches the difference in the *d*-orbitals.

STUDENT LAB MANUAL NOTES FOR EXPERIMENT

This experiment will take place over two days, and will involve:

First day: the extraction of acetylsalicylic acid from commercial aspirin and complexation reaction of the extracted aspirin in mild conditions;

Second day: photophysical studies and characterization of the obtained compound in solution. All sections of this experiment are to be completed in group of 3-4 students.

1. LEARNING OUTCOMES

After undertaking this experiment, you will achieve the following laboratory skills:

- Solvent extraction;
- Recrystallization;
- Vacuum filtration;
- Thin layer chromatography;
- Metal complexation equilibria;
- Mass spectrometry (ESI and ESI-MS/MS) and Mass spectra simulation;
- IR, UV-vis, Fluorescence spectroscopy.

Crystal field theory demonstration and visualization and discussion about the modification of the energy in the *d*-orbitals during the complex formation.

Other improvements in understanding crystal/ligand field theory, acid - base equilibrium and hydrolysis, reaction with an organic compound (aspirin or salicylic acid) and different transition metal ion, as well as the colors of some complexes of iron, copper ion and verify the presence of different compound in a commercial drug.

2. PROCEDURE

SAMPLING AND EXTRACTION

The extraction and purification of acetylsalicylic acid from commercial aspirins

- Weigh 2 tablets (about 1-1,50 g) of aspirin in beaker.
- Add 10 ml warm ethanol or acetone and stir for 30 min.
- Filter in Buchner using glass wool.
- Collect the filtrate together. (If there is any impurities re-filter).
- Add water (1ml) at the filtrate and the solution will be cooled slowly and then chilled.
- Repeat crystallization.

Fe(III) COMPLEX

- Fill test tubes with 2.5ml of 10^{-2} M metal ion water solutions (Fe(III), Co(II), Ni(II), Cu(II), Zn(II)) and add 1 drop of HCl 1M.
- Employ the collected acetylsalicylic acid as a solution (2.5ml, 10^{-2} M) for reaction with acid metal ions solutions (Fe(III), Co(II), Ni(II), Cu(II), Zn(II)).
- Observe any color variations.

Cu(II) COMPLEX

- Dissolve the previously purified acetylsalicylic acid (about 1g) in aqueous solution of NaHCO₃ (10%) 12ml.
- Filter the resulting solution to remove any undissolved compound.
- Add a solution of Cu(II) (Cu(II) nitrate, acetate or sulfate are suitable) (about 350mg in 10 ml of water).
- Bright blue crystals of copper aspirinate immediately form and precipitate.
- Collect the filtrate together for the analysis.

3. REPORT

In the results section of your report, you should include the following:

- A description of the appearance of your solutions;
- The yield of your extraction of aspirin, and the percentage yield, showing your working;
- The IR, UV-vis, fluorescence, ESI and ESI-MS/MS spectra of your ligand and complexes, with comparison to a reported literature;

- A narrative tying together the various results reported here.

In the discussion section of your report, you should include answers to the following:

Discuss the binding stoichiometries of the Fe and Cu complexes, suggesting reasons for the difference or similarity between the two complexes;

Using ChemDraw, or a similar structure-drawing software, propose the structure of the complex that you synthesized

Using a free mass spectra simulator (as: <http://www.chemcalc.org>), simulate every compound that you have synthesized and compare the simulated spectrum to the experimental one.

WORK INSTRUCTIONS FOR SERVICE ROOM

Based on a group of 15 students each lab session

Chemicals

150 g aspirin tablet

1L ethanol

50 g copper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) or copper(II) sulfate pentahydrate ($\text{Cu}(\text{SO}_4)_2 \cdot 5\text{H}_2\text{O}$)

50 g iron(III) chloride (of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)

500g sodium bicarbonate (NaHCO_3)

1L dichloromethane

10 TLC sheets

100ml of HCl 1M

Fume hoods

Each fumehood (x9) should have 2 setups. Each setup should contain:

2x 100 mL beaker

1x 100 mL conical flask

1x 50 mL measuring cylinder

1x 250 mL filter flask

1x Buchner or Hirsch funnel

Steam bath

Communal lab space

2 tray reagents containing aspirin tablet, ethanol, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, FeCl_3 , NaHCO_3 (Beside each balance)

9x ethanol squirt bottles (Place one per fumehood)

2x preparative balance (One at the end of Bench A & Bench C)

Small tray of plastic weigh boats (beside each balance)

Tray of spatulas (one beside each balance)

Tray of heat gloves (End of bench A or C)

2x boxes of filter paper (beside each balance)

8x acetone wash bottles (2 per sink)

4x "non-halogenated organic waste" 5L bottles with ECOfunnels (1 per sink)

2x "iron(III) waste" 5L bottles (1 every 2nd sink)

2x "copper(II) waste" 5L bottles (1 every alternate 2nd sink)

Instrument and Equipment Preparation

Cleaning/Preparation of Workstations/Instrument Rooms

(The amount of daily cleaning is dependent on staff and time constraints; not all of the following may be possible every day. The order is in decreasing priority.)

- Check for missing/broken/obviously dirty glassware; replace/clean
- Refill ethanol and dichloromethane squirt bottles
- Restock chemicals beside balance
- Wipe any obvious spills on benches/fumehoods