الجامعة الألمانية الأردنية
German Jordanian University

School of Medical Sciences

Pharmaceutical and Chemical Engineering Department

Laboratory Manual

for

Instrumental Analysis

PCE372

Edition: 2021

Contents

GENERAL LABORATORY INSTRUCTIONS

Attendance

- Laboratory attendance is **MANDATORY (NOT optional),** unless special arrangements are made with your instructor (no more than two sessions), accompanied by a **valid excused absence.** There will be **no make-up** laboratories.
- Students are not allowed to leave the laboratory during the session without obtaining permission.

Laboratory notebook

- The week's experimental procedure are available in the lab manual.
- Students are required to read carefully the experimental procedure before coming to the lab.
- The lab report with completed lab work should be submitted to the lab TA at the beginning of the next lab session in order to be signed and graded, note that the data sheet should have the instructor signature.

Lab Safety Lecture

- Each student must wear a clean, white laboratory coat at all times while in the laboratory. And also, they should wear Safety Goggles, Closed-Toed Shoes, Clothing that covers your legs, do not wear contact lenses

- All equipment and bottles should be returned to the proper place after use.
- Assume all chemicals used in the experiment are dangerous.
- Eating or drinking in the laboratory is prohibited.
- Do not pipette by mouth or carry reagents around the lab.
- Wash your hands thoroughly before leaving the lab.

LABORATORY STRUCTURE

- Students will work in groups.
- Students are required to read the theory and procedures of each experiment before coming to the lab.

- There will be a brief lecture-discussion on the theory and procedure before each new experiment
- After the completion of each experiment, one full report must be submitted for each experiment to the TA at the beginning of the next lab.

Assignment of Grades

Reports	**…… 40%**
Quizzes	**…… 10%**
Evaluation	**…… 10%**
Final Exam	**…… 40%**
Total	**…… 100%**

Guidelines for Writing a Laboratory Report

Each group should prepare a formal laboratory report which consist of the following

Title page

Should include the title of the experiment, course name, name of students, date of work and date of submission

Abstract

The Abstract is an informative summary of what you did and what you found out. In addition, it is less than 200 words.

The Abstract should include the following:

- Objectives (as outlined in the Introduction) and scope of the investigation.
- A summary of the results and conclusions - a brief but thorough statement of the outcome/s of the experiment.

Although the Abstract comes first in a report, it is best to write it last, after you have the results and conclusions.

Introduction

This provides a summary of the analysis to be undertaken. The purpose of the Introduction is to put the reader in the picture and place the research/experiment within a context. The Introduction is the what and why of the experiment, and should answer the following questions:

- What was the purpose or objective of the experiment/research?
- What was the hypothesis (an idea or concept) that can be tested by experimentation if there is one?
- Why was it important in a broader context?

The Introduction should not include any results or conclusions, and should contain one or tow paragraph.

Experimental

Experimental, is a description of the materials and procedures used - what was done and how. Describe the process of preparation of the sample, specifications of the instruments used and techniques employed.

While the Method does not need to include minute details (e.g. if you followed a set of written instructions, you may not need to write out the full procedure - state briefly what was done and cite the manual), there needs to be enough detail so that someone could repeat the work.

Don`t copy the procedure in the manual

Results

This section states what you found; include only your own observed results in this section.

The following will be included in your Results:

- Pictures and spectra.
- Tables and graphs whenever practical.
- Provide sample calculations for key elements of the lab: dilutions, standard curve use, etc.
- Brief statements of the results in the text (without repeating the data in the graphs and tables). When writing about each picture, graph or table, refer to it parenthetically e.g. (Figure 1).

Discussion

State your interpretation of your findings, perhaps comparing or contrasting them with the literature. Reflect on your actual data and observations.

Explain or rationalize errant data or describe possible sources of error and how they may have affected the outcome.

The Discussion must answer the question "What do the results mean?" It is an argument based on the results.

Conclusion

This is the summing up of your argument or experiment/research and should relate back to the Introduction.

The Conclusion should only consist of a few sentences and should reiterate the findings of your experiment/research.

If appropriate, suggest how to improve the procedure, and what additional experiments or research would be helpful.

References

Cite any references that you have used, ensuring that each item in the reference list has an in-text citation, and every in-text citation has a full reference in the reference list at the end of your paper.

Grades for each section

Title page	**……. 2**
Abstract	**……. 10**
Introduction	**……. 10**
Experimental	**……. 10**
Results	**……. 30**
Discussion	**……. 30**
Conclusion	**…….. 6**
References	**…….. 2**
TOTAL	**…….. 100**

Experiment 1

Spectrophotometric Analysis of a Commercial Aspirin Tablet

Objective:

The purpose of this Experiment is to determine the amount of aspirin in a commercial aspirin product.

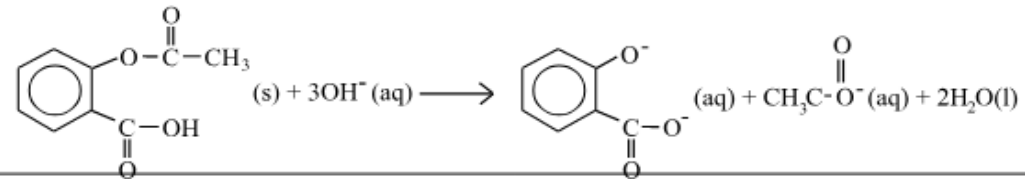
Introduction:

Acetyl salicylic acid (ASA) is one of the oldest synthetic drugs. First synthesized in Germany by the Bayer Company and marketed under the name "Aspirin" it has remained one of the most popular "over the counter" drugs of all time. Its main effect is as a painkiller and fever depressant, but in addition, there is strong evidence that in low daily dosages it lowers the incidence of heart attacks. In the last few decades, other drugs such as acetaminophen (commercial trade name Panadol, also Tylenol) and ibuprofen (trade name Advil) have taken much of the market for ASA, but ASA remains an important and widely used medicine. Drugs, in addition to their active compound, often contain other inactive ingredients (called excipients in the pharmaceutical industry) such as binders, fillers, dyes, drying agents, etc. The content of active ingredient in a tablet will always be stated on the package. In this experiment, we will determine the percent active compound in a commercial aspirin tablet. Aspirin is the trade name for acetylsalicylic acid (ASA). The ASA in the tablet will be reacted with Fe^{3+}, forming an intensely violet colored complex. The concentration of the complex will be determined by means of spectrophotometry, using a UV/VIS spectrophotometer. Finally, we will be able to calculate the weight and the weight% of ASA in the commercial tablet.

<u>The determination of acetyl salicylic acid by spectrophotometry</u>

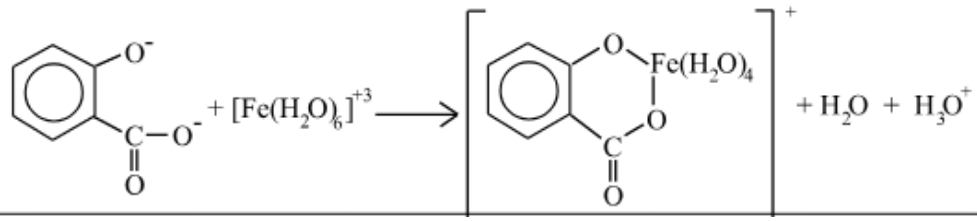
Acetylsalicylic acid is the acetate (ethanoate) ester of salicylic acid, 2-hydroxybenzoic acid. The "acetyl" ester is rapidly hydrolysed to the salicylate anion in basic medium, as shown in the following reaction.

The complex is formed by reacting the aspirin with sodium hydroxide to form the salicylate dianion.



Once the de-esterification is complete, the solution is acidified, and $FeCl_3$ is added. The salicylic acid will react with the Fe^{3+} to form a colored complex ion:

The addition of acidified iron (III) ion produces the violet tetraaquosalicylatroiron (III) complex.



The spectrum of the Fe salicylate complex was shown in figure 1, plotted as T vs. wavelength. Note that the lowest transmission (i.e., the largest absorption of light) is in the blue green and yellow areas of the spectrum, resulting in the violet color. Maximum absorption (minimum transmittance) is just above 500 nm, and the concentration measurement will be done at this wavelength.

In order to calculate the concentration of the complex we would need to know the value of ε and l. Instead, we will measure the absorbance of Fe-salicylate complex solutions of known concentration and plot the absorbances of a number of such known solutions vs. the concentration. This is known as a calibration curve. The calibration solutions are prepared by first making a solution of the Fe-salicylate complex of known concentration. This solution is called the stock solution. Next, we make 5 standard solutions by diluting a known amount of stock solution. The Absorbance of each of these 5 solutions is measured, and plotted vs. their concentration resulting in a linear calibration curve of A vs C. Next, we measure the absorbance of the solution prepared from the commercial aspirin solution and find its concentration by comparing its absorbance value on the calibration curve.

Procedure

Stock solution

- In this experiment, you need to prepare 1.0 M of NaOH and 0.02 M $FeCl_3$ (buffered to pH = 1.6 with HCl/KCl)
- The buffer solution can be prepared by mixing 250 mL of 0.2 M KCl and 131.5 mL of 0.2 M HCl and dilute them to 1L with Distilled water.

Preparation of Standard solutions

- Weigh about 0.160 g Salicylic acid directly into small dry beaker. Record the mass (g). Dissolve it in 5 mL NaOH. Heat on hot plate until dissolved. Then cool the solution.
- Transfer solution to 100 mL volumetric flask. Fill to the mark with distilled water. This is your "stock" Salicylic acid solution
- Prepare five standard solutions: use micropipette to add 250, 500, 750, 1000 and 1250 µL stock SA into 25.0 mL volumetric flasks. Fill each flask to the mark with buffered $FeCl_3$ solution. Solutions will vary from light to dark violet color.
- Measure and record the absorbance of each solution at 530 nm, using the $FeCl_3$ solution as blank.

Aspirin Sample

- Weigh Aspirin tablet in beaker; record the mass, crush tablet. Dissolve it in 5 mL NaOH. Heat on hot plate until dissolved (some fine non-active ingredients may still be visible). Cool.
- Filtrate the solution and Transfer it to 100 mL volumetric flask. Fill to the mark with distilled water. This is your "stock" Aspirin solution
- Prepare one Aspirin solution: use micropipette to add 750 µL Aspirin stock in 25.0 mL volumetric flask. Fill the flask to the mark with buffered $FeCl_3$ solution. The solution will have a medium dark violet color.
- Measure and record the absorbance at 530 nm

Experiment 2

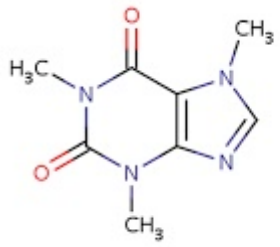
UV Spectroscopic Analysis of Caffeine and Benzoic Acid in soft drink

Objective:

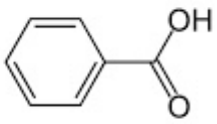
To measure the concentration of caffeine and benzoic acid in soft drinks using uv-vis spectrophotometer

Introduction:

In this experiment, we use ultraviolet absorbance to measure two major species in soft drinks. Caffeine is added as a stimulant and sodium benzoate is a preservative. Benzoic acid is a food preservative and it is widely used in acidic foods (pH 2.5 - 4). It is normally added as the salt, sodium benzoate. Although benzoic acid is a man-made food additive, benzoic acid naturally occurs in several fruits, often in amounts that exceed the recommended allowance set by the FDA.



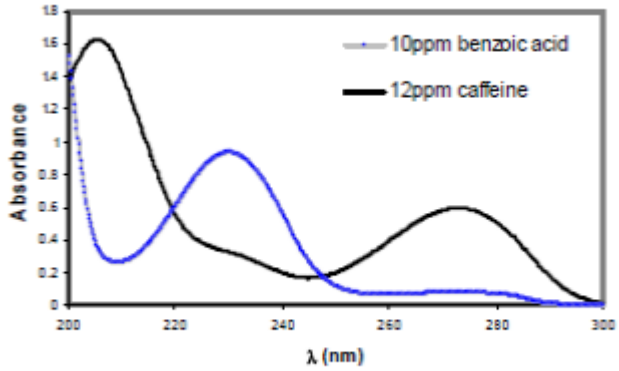
Caffeine



Benzoic Acid

In this analysis, we will use non-diet soft drinks because the sugar substitute, aspartame, found in diet drinks, also absorbs ultraviolet radiation. This slightly interferes in the analysis. We also avoid drinks containing carmelized sugar, as this colorant absorbs in the UV region of interest. Therefore, in this experiment we shall analyse Mountain Dew, a highly caffeinated soft drink, and Sprite, which does not contain caffeine. There will be some UV absorbance from the matrix of these drinks. This does cause a small systematic error, but this error is very small compared to the levels of caffeine and benzoic acid being measured.

As seen in the spectrum below, both caffeine and benzoic acid absorb UV light at many of the same wavelengths.



UV absorption of benzoic acid and caffeine in 0.01 M HCl

Beer's law can be applied to solutions containing more than one kind of absorbing substance. Provided there is no interaction among the various species (side reactions), the total absorbance for a multicomponent system given by:

$A_{total} = A_1 + A_2 + \ldots + A_n$ (Equation 1)

$A_{total} = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \ldots + \varepsilon_n bc_n$

Where the subscripts refer to absorbing components 1, 2 …n.

The above equation indicates that the total absorbance of a solution at a given wavelength is equal to the sum of the absorbances of the individual components present. This relationship makes possible the quantitative determination of the individual constituents of a mixture, even if their spectra overlap. If enough spectrometric information is available, all of the components of mixtures can be quantified without separation. For a two-component mixture (compound X and Y) with overlapping absorbances, you can solve for the concentration of each species, [X] and [Y], by measuring the absorbances at two different wavelengthts, λ' and λ". The problem is mathematically equivalent to having two simultaneous equations with two unknowns.

$A1 = \varepsilon_{x,1} bcx + \varepsilon_{Y,1} bcY$ (total absorbance at λ') (Equation 2)

$A2 = \varepsilon_{x,2} bcx + \varepsilon_{Y,2} bcY$ (total absorbance at λ") (Equation 3)

The four molar absorptivities, $\varepsilon_{x,1}$, $\varepsilon_{y,1}$, $\varepsilon_{x,2}$, $\varepsilon_{y,2}$, can be evaluated from individual standard solutions of X and Y, or better, from the slopes of their Beer's law plots. The problem becomes simpler when one of the compounds has no interference with the other compound. If there is substantial interference, then you must solve the simultaneous equations.

Using UV spectroscopy, you will determine the concentrations of caffeine and sodium benzoate (Determined as benzoic acid), in the soft drink Mountain Dew. The UV spectra of caffeine and benzoic acid overlap at certain wavelengths, thus you will need to measure the absorbance of the unknown mixtures using two different wavelengths and apply equations 2 and 3 to evaluate the concentrations of caffeine and benzoic acid.

Procedure

Stock solution

- In this experiment, you need to prepare two stock solutions benzoic acid (10mg/L) and caffeine (20mg/L) solutions in a 0.010M solution of HCl.

Calibration Curve Standards

- Prepare a set of benzoic acid solutions containing 2, 4, 6, 8, and 10 mg/L in 0.010 M HCl.
- Prepare caffeine standards containing 2, 4, 6, 8, & 10 mg/L in 0.010 M HCl.

Soft Drink Samples

- Warm ~ 20 mL of soft drink in a beaker on a hot plate to expel CO2. (Do not boil!)
- Filter the warm liquid through filter paper to remove any particles.
- After cooling to room temperature, pipette 2.00 mL into a 50-mL volumetric flask.
- Add 0.010 M HCl up to mark.

The Absorbance

- Measure the ultraviolet absorption spectrum of the standards and the diluted soft drink samples at 230 and 270 nm.

Experiment 3

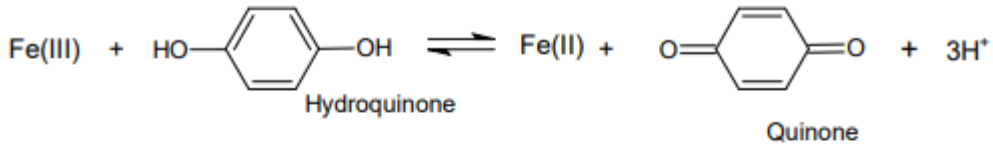
Spectrophotometric Determination of Iron in Vitamin Tablets

Objective:

To measure the amount of iron in supplement tablet sing standard addition method and uv vis spectrophotometer

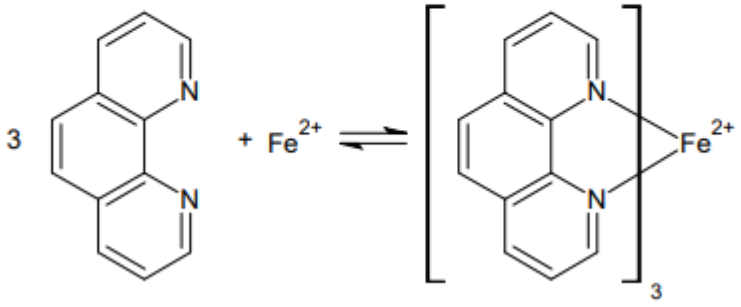
Introduction:

In this Experiment, iron from a vitamin supplement tablet is dissolved in acid, reduced to Fe^{2+} with hydroquinone, and complexed with o-phenanthroline to form an intensely colored complex using standard addition method.



Hydroquinone

Quinone



o-phenanthroline

The standard additions method (often referred to as "spiking" the sample) is commonly used to determine the concentration of an analyte that is in a complex matrix such as biological fluids, soil samples, etc. The reason for using the standard additions method is that the matrix may contain other components that interfere with the analyte signal causing inaccuracy in the determined concentration. The idea is to add analyte to the sample ("spike" the sample) and monitor the change in instrument response. The change in instrument response between the sample and the spiked samples is assumed to be due only to change in analyte concentration.

Procedure

Stock solution

- In this experiment, you need to prepare
- 100 mL of 6M HCl
- O-phenanthroline 0.25 g in 10 mL ethanol and 90 mL water **(sonication)**
- Hydroquinone 1g in 100 mL water (amber bottle)
- $(NH_4)_2$ Fe $(SO_4)_2.6H_2O$: 0.07 g per 250 mL (1mL of conc. HCl)
- Sodium Citrate: 2.5 g per 100 mL H_2O
- Unknown solution: dissolve the tablet with 50 mL of 6 M HCl, heat to boil, filtrate the solution and then complete the dilution up to 100 mL, then pipet 5 mL of the solution and dilute it again to 100 ml using distilled water.

Calibration Curve Standards

- Prepare the solutions in the following table (the volumes are in mL)

No.	Unknown	Hydroquinone	Sodium Citrate	Standard Fe	O-Ph	H2O
blank	0	0	15	0	10	
1	2.5	5	15	0.5	10	
2	2.5	5	15	1.0	10	Up to
3	2.5	5	15	1.5	10	50mL
4	2.5	5	15	2.0	10	
5	2.5	5	15	2.5	10	

The Absorbance

- Allow the solution to stand for at least 10 min, then measure the absorbance for each solution at $\lambda = 510$

Experiment 4

Spectrophotometric Determination of Paracetamol in Pharmaceutical formulations

Objective:

The purpose of this experiment is to determine the concentration of paracetamol in a tablet using uv-vis spectroscopy

Introduction:

Paracetamol (acetaminophen or N-acetyl-4-aminophenol), herein referred to as PA, is a popular antipyretic and analgesic agent. In several countries, it is one of the most used medicines as an alternative to aspirin (acetylsalicylic acid). For PA determination in drug formulations, different methods exploiting.

In this experiment, we describe a simple, selective and precise method for rapid spectrophotometric determination of paracetamol in pharmaceutical formulations. In the method, cerium (IV) was reacted for 60 min with paracetamol in concentrated sulphuric acid in a water bath maintained at 80 °C. The final product of the reduction of cerium (IV) is cerium (III), which can be followed by spectrophotometrically (λ+ 255 nm - 348 nm).

This method was based on the oxidation reaction of paracetamol with cerium (IV) in sulphuric acid media. At higher concentrations of sulfuric acid, the redox potential of cerium (IV) is such that it can be oxidized. This indicates that de-acetylation of paracetamol to p-aminophenol (Fig.1) is the rate-determining step. P-aminophenol is then further oxidized with Ce (IV) to p-aminoquinone

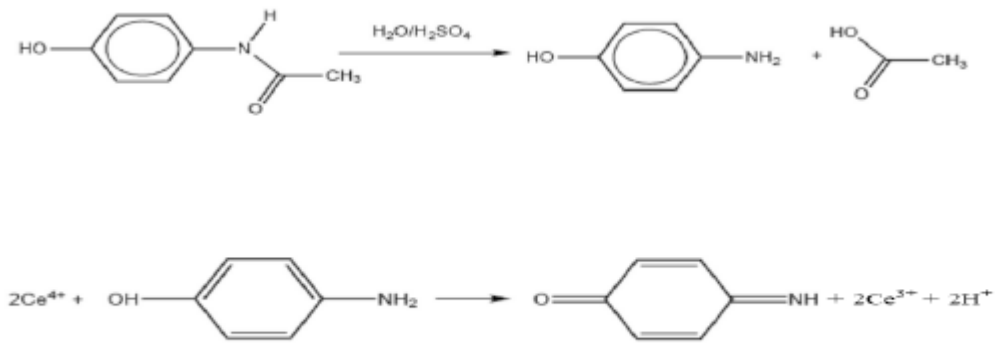


Fig. 1. de-acetylation of paracetamol to p-aminophenol

Procedure

Preparation of Standard solutions

- 3.5 M H_2SO_4.
- 0.025 M $K_2Cr_2O_7$

Aspirin Sample

- Weigh Panadol tablet; record the mass, crush the tablet. Dissolve it in 100 mL 3.5 M H_2SO_4.
- In reflux system heat the solution for one hour keep the temperature between 60º and 80º C (avoid boiling).
- Filtrate the solution, pipette 5.00 mL of the filtrate, and dilute it to 25.0 mL using distilled water
- Prepare the blank by dilute 5.00 mL of 3.5 M H_2SO_4 to 25.0 mL using distilled water.
- Read the absorbance (scan range 400 -700) for the prepared solution, then add 0.50 mL of 0.025 M $K_2Cr_2O_7$ and again measure the absorbance.
- Return the sample to the solution flask and repeat the addition of 0.50 mL of 0.025 M $K_2Cr_2O_7$ and the reading of the absorbance until you get the maximum absorbance and then the decreasing in it

Experiment 5

Spectrophotometric Determination of Sulphamethoxazole in Pharmaceutical Formulation

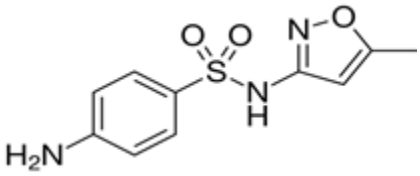
Objective:

The purpose of this lab is to determine the concentration of Sulphamethoxazole in a tablet using Fluorescence spectroscopy

Introduction:

Sulfamethoxazole is antibacterial agents that used to treat bacterial infections. Its pharmaceutical formulation is an important progress in the development of antimicrobial agents (anti-biotics).

Sulfamethoxazole is bacteriostatic which means they inhibit the growth of bacteria but do not kill them. This active pharmaceutical ingredient is an active ingredient in anti-biotic that used in the treatment of UTI, chronic bronchitis, meningococcal meningitis. Those products are usually mixed with another drug to increase their power.



In this experiment, we describe a simple and precise method for rapid spectrophotometric determination of Sulfamethoxazole in pharmaceutical formulations

Procedure

Stock solution

- Briton-Robinson buffer
  Prepared by adding 2.90 mL of CH_3COOH (17.4M), 3.40 mL of H_3PO_4 (14.8M) and 3.10 g of H_3BO_3 into 500 mL Volumetric flask complete it to the mark with Distilled water
- 100 mL of 5 M NaOH

Preparation of Standard solutions

- Check the pH for the Briton-Robinson buffer and make it around 2 using 5 M NaOH
- Prepare 100 mL of $2.5x10^{-3}$ M of Sulphamethoxazole using Briton-Robinson buffer for dilution.
- Prepare 5 different standards by pipetting 250, 500, 750, 1000, 1250µL of the stock solution in 25mL volumetric flasks, filling it to the mark with the modified Briton-Robinson buffer.

Tablet Sample

- Weigh Balkatrin tablet in beaker; record the mass, crush tablet. Dissolve it in 100 mL volumetric flask using Briton-Robinson buffer.
- Filtrate the solution and pipette 500µL to 25 mL volumetric flask. Fill to the mark with modified Briton-Robinson buffer.

Measuring the standards and the sample

- Measure the fluorescence of the standards and the tablet sample using the following conditions:
  - 260 nm for excitation
  - 365 nm for emission
  - 5 mm for entrance and exit slits

Experiment 6

Potentiometric titration of Iron tablet

Objective:

To measure the amount of iron in a tablet using potentiometric titration methods.

Introduction:

In a potentiometric titration, the equivalence point is detected by monitoring the electromotive force (e.m.f.) of an electrochemical cell formed by an indicator electrode coupled with a convenient reference electrode. The potential of the indicator electrode, and hence the e.m.f. of the cell, is a measure of the activity (approximately the concentration) of the ionic species in the electrolyte solution.

The potentiometric redox titration performed here involves the oxidation of iron(II) by the oxidising agent cerium(IV) according to

$Fe^{2+}_{(aq)} + Ce^{4+}_{(aq)}$ S $Fe^{3+}_{(aq)} + Ce^{3+}_{(aq)}$

The potential of a platinum indicator electrode in contact with a solution of these ions will clearly vary as the titration proceeds. To monitor these changes, a second reference electrode (here $Ag_{(s)}$ Ω $AgCl_{(s)}$ Ω $Cl^-_{(aq)}$) is immersed in the solution to complete the electrical circuit. The cell can be expressed in terms of the Fe^{3+}, Fe^{2+} couple

$Ag_{(s)}$ _ $AgCl_{(s)}$ _ $KCl_{(aq,3M)}$ $Fe^{3+}_{(aq)}$, $Fe^{2+}_{(aq)}$ _ Pt

later in terms of the Ce^{4+},Ce^{3+} couple

$Ag_{(s)}$ _ $AgCl_{(s)}$ _ $KCl_{(aq,3M)}$ $Ce^{4+}_{(aq)}$, $Ce^{2+}_{(aq)}$ _ Pt

The dependence of the potential of the indicator electrode on the activities of the various ionic species in solution is given by the following equivalent Nernst equations

$$E_{Fe^{3+},\,Fe^{2+}} = E_{Fe^{3+},\,Fe^{2+}} + \frac{RT}{F} \ln \frac{a_{Fe^{3+}}}{a_{Fe^{2+}}} \qquad (1a)$$

$$E_{Ce^{4+},\,Ce^{3+}} = E_{Ce^{4+},\,Ce^{3+}} + \frac{RT}{F} \ln \frac{a_{Ce^{4+}}}{a_{Ce^{3+}}} \qquad (1b)$$

The cell e.m.f. is thus expressible as:

$$E = E_{Fe^{3+},\,Fe^{2+}} - E_{Ag\,|\,AgCl\,|\,Cl^-}$$

$$= E_{Fe^{3+},\,Fe^{2+}} + \frac{RT}{F} \ln \frac{c_{Fe^{3+}}}{c_{Fe^{2+}}} - E_{Ag\,|\,AgCl\,|\,Cl^-} \qquad (2a)$$

$$E = E_{Ce^{4+},\,Ce^{3+}} - E_{Ag\,|\,AgCl\,|\,Cl^-}$$

$$= E_{Ce^{4+},\,Ce^{3+}} + \frac{RT}{F} \ln \frac{c_{Ce^{4+}}}{c_{Ce^{3+}}} - E_{Ag\,|\,AgCl\,|\,Cl^-} \qquad (2b)$$

in which, for the sake of simplicity, ionic activities have been replaced by ionic concentrations.

Procedure

Stock solution

- 100 mL of 6M HCl
- 100 mL of 20mM Cerium sulphate in 50 mL and 50 ml 6 M HCl

Preparation the sample

- prepare the titration setup and filtration setup
- Weigh one of nutrients capsules and crush it.
- put the tablet inside a E.M flask (100ml)
- Add 50 mL 6M HCl to it.
- place the flask on the hot plate at 100 ºC in order to dissolve capsule (using thermometer)
- Using the filtration setup to filtrate the solution by gravity.
- take the filtrate and react it with zinc inside a buret then take 25 mL of the product
- Make sure to rinse the buret with (Ce 4+) before using it for titration
- Titrate it with 50 mL of 20 mM ceriumsulphate by adding 1 mL each time and measure the value of the potential.

Experiment 7

Titration of Phosphoric Acid and Acetic Acid with NaOH

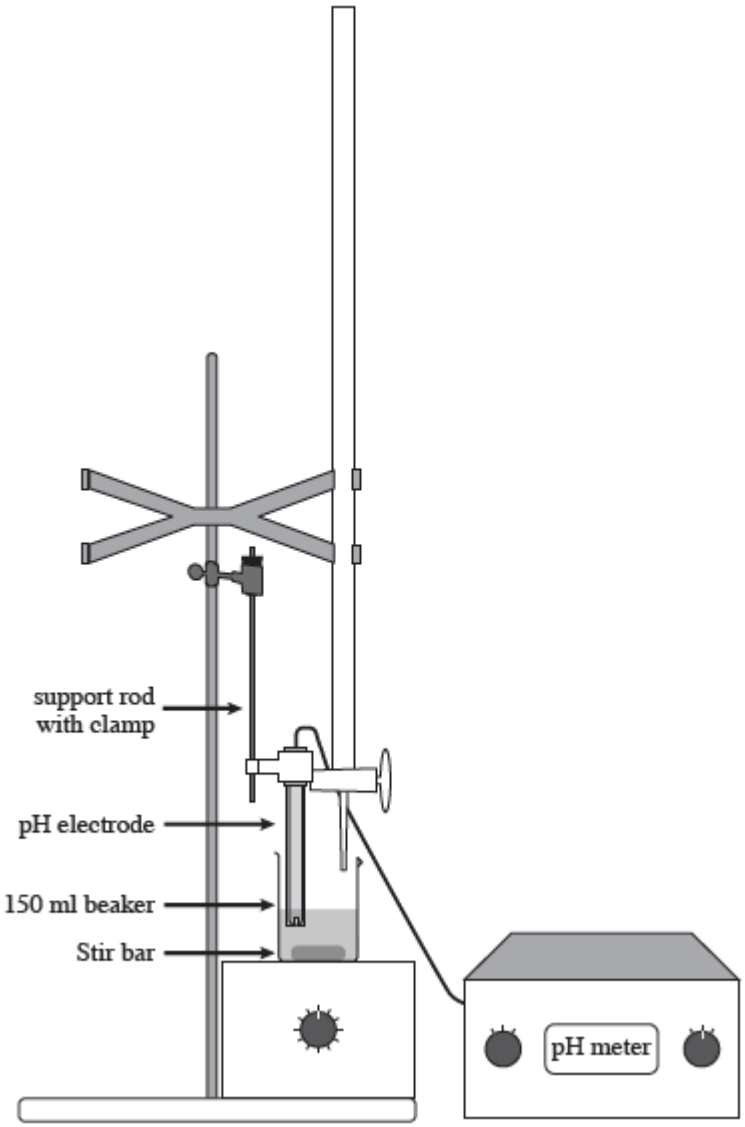
Objective:

To find the equivalence point for monoprotic and polyprotic acids using pH meter

Introduction:

Bases react with acids. OH- is a strongly basic ion, and NaOH is the most common strong base. When the generic acid, HA, reacts with NaOH, the reaction is:

Phosphoric acid, H3PO4, is a polyprotic ("many hydrogen") acid. You will react sodium hydroxide with this acid while carefully monitoring the reaction for indications that stoichiometric quantities of the reactants have been used. "Stoichiometric" means that the quantities of reactants equal the amounts required in balanced chemical equations. "Carefully monitoring" means to repeatedly add one reactant in small quantities while watching for some physical sign that shows that the proper stoichiometric amount has been added. This process is called "titration". In this experiment, the reactants are dissolved in water. The solutions have been carefully prepared and their concentrations are marked on the labels. You will monitor the quantities of reactants used in the reaction by measuring the volumes of the solutions used. Two volumetric measuring tools will be used: a buret and a pipe. You will monitor the moment when the reaction has reached stoichiometric quantities of reactants using two different indicators and a pH meter. An "indicator" is a chemical that changes color as conditions in the solution change. You will use two different acid-base indicators that change color as the pH of the solution changes. Methyl orange changes from red to yellow as the pH changes from 3 to 5. Phenolphthalein changes from colorless to pink as the pH changes from 8 to 10. The "pH meter" is an instrument that measures the pH of a solution by checking the voltage output of a special battery (the pH electrode) which puts out various voltages in proportion to the pH of the solution surrounding the immersed end of the battery.


support rod
with clamp
pH electrode
150 ml beaker
Stir bar
pH meter

Procedure

Stock solution

- 100 mL of 0.1M NaOH
- 50 mL of 0.1M Phosphoric acid
- 50 mL of 0.1M Acetic acid

Preparation the sample

- prepare the titration setup
- standardize the NaOH solution using 0.2 g KHP (2 trials)
- pipet 10 mL of the Phosphoric acid solution and put it in 150 mL beaker
- add 25 ml of distilled water
- measure the pH for the solution then titrate the solution with the standardized NaOH solution measure the pH after each 0.5 mL you add
- complete the addition until you reach pH = 12
- repeat the procedure using acetic acid

Experiment 8

Quantitative Analysis of Ascorbic Acid Using Voltammetry

Objective:

In this experiment, you will be introduced to cyclic voltammetry techniques to quantify the ascorbic acid content in an unknown solid.

Introduction:

Electrochemistry comprises a group of important analytical techniques that can provide quantitative information on the composition of an analyte as well as information about standard reduction potentials and rates of electron transfer following chemical reactions. Electrochemical detectors are also becoming popular for chromatographic separations. The variety of information that can be obtained as well as the relatively low cost of the equipment and the convenience of the experiments, make electrochemical techniques a very versatile and widely used for both qualitative and quantitative chemical analysis.

There are three main types of electrochemical experiments: potentiometry, coulometry, and voltammetry. Potentiometry is the measurement of the potential of a solution under conditions of low current flow. pH and other ion selective electrodes are common examples of the use of potentiometry to quantify species in solution. Coulometry involves the quantitative conversion of an analyte via the transfer of electrons. The total charge or current is monitored as a function of time in a coulometry experiment in order to quantify the amount of analyte converted. Voltammetry involves the measurement of the current at an electrode as a function of the applied potential. Although more commonly used for studies of redox potentials and kinetics, special forms of voltammetry can be effectively used for the quantitative analysis of electroactive species. Polaragraphy and stripping voltammetry, using a mercury drop electrode, are common methods.

In this experiment, you will use voltammetry with a printed-screen carbon electrode to determine the percent of the ascorbic acid in a solid unknown.

Ascorbic acid, or vitamin C, is an important nutrient. Various methods exist for quantifying ascorbic acid in a variety of food. Although ascorbic acid absorbs light in the UV region, spectrophotometric methods for the determination of ascorbic acid in food often suffer from interference due to absorbance by other components. Acid-Base titration methods are also not very selective and have rather poor detection limits. Redox titration using triiodide and starch as an indicator is a reliable method to quantify the amount of ascorbic acid in a solution. Chromatography is commonly used with a variety of detectors including UV and electrochemical. Ascorbic acid is easily oxidized to dehydroascorbic acid, which undergoes further chemical reaction to form the gem-diol. The oxidation potential is pH dependent. In this experiment the electrochemical reaction can be induced and monitored by voltammetry to quantify the concentration of ascorbic acid in solution.

Procedure

Stock solution

- Prepare a phosphate buffer of PH equals 2, by adding 0.125g of Na_2EDTA to distilled water and letting it to dissolve on the stirrer, then add 0.7 ml of H_3PO_4 (stock) and again ensure that the solution is homogenous and finally adding 1.57g of KH_2PO_4 and fill it to 250ml mark with distilled water.
- Prepare ascorbic acid solution by adding 0.088g of ascorbic acid and fill it to 100ml mark with the phosphate buffer previously made.
- Prepare unknown solution by adding 0.20g of the unknown (Tang) and fill it to 100ml mark with the phosphate buffer previously made.

Preparation the sample

- Purge the standard and the unknown for 5 minutes by nitrogen to remove oxygen.
- Then place 25 ml from the unknown in the voltammetry cell and then add 2 mL of the standard to the unknown. Measure the current in each addition

Experiment 9

Thermal gravimetric analysis Determination of Calcium in vitamin tablet

Objective:

To determine the amount of calcium in vitamin tablet using TGA instrument.

Introduction:

Thermal gravimetric analysis (TGA) is a thermal characterization method that measures the mass of a sample over time as the temperature increases at a determined rate. The main use is to detect chemical based changes that include decompositions of volatiles, solid-gas reactions and chemisorption's based on temperature. The instrumentation itself is rather basic and is called a Thermogravimetric Analyzer. It is equipped with a furnace that has a precision balance with a sample pan and programable control for temperature that enables precision heating ramps. The temperature is normally increased at a constant rate to induce the thermal reactions. The atmosphere within the furnace can be changed based on the experimental conditions required for testing. The input gas into the furnace can be ambient air, Nitrogen or gases that provide an oxidizing or reducing environment to determine how the reaction of the samples changes.

Calcium oxalate monohydrate ($CaC_2O_4.H_2O$) is commonly used in ceramic glazes for industrial uses, and is the common component of kidney stones in the human body. Many laboratories and universities will use this material as a standard or calibration material before running other TGA samples. It undergoes 3 specific weight losses that are separated by enough temperature to easily identify each of the 3 decomposition products, as shown by the chemical reactions below:

$CaC_2O_4.H_2O$ (s)

$\Delta \rightarrow CaC_2O_4$ (s) + H_2O (g) (1)

$\Delta \rightarrow CaCO3\ s + CO\ (g)$ (2)

$\Delta \rightarrow CaO\ s + CO2\ (g)$ (3)

Each of these reactions has a decomposition byproduct of water, carbon monoxide and carbon dioxide, respectively

Procedure

Stock solution

- 100 mL of 6% (w/v) $(NH_4)_2C_2O_4$
- 100 mL of 1 M HCl

Preparation the sample

- Weigh your tablet and crush it.
- Weigh 1 g sample of your calcium-containing sample into 250-mL E.M flask.
- Add 100 mL of (1 M) HCl.
- Heat the solution to boiling.
- Filtrate your sample.
- Add 50 mL of hot 6% (w/v) $(NH_4)_2C_2O_4$ solution on the filtrate.
- Precipitate CaC_2O_4 by slowly adding NH_3. In addition, constantly measuring the pH using litmus paper. (PH 4.5 to 5.5). Allow the solutions to stand for 5 min.
- Weigh filter paper.
- Filter the precipitate and put it in the oven to dry.
- Use TGA instrument.

Experiment 10

Quantitative Analysis of paracetamol in tablet Using HPLC

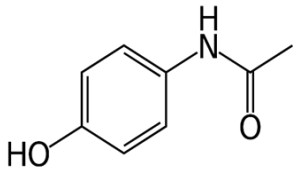
Objective:

To determine the concentration of Paracetamol in a tablet using HPLC

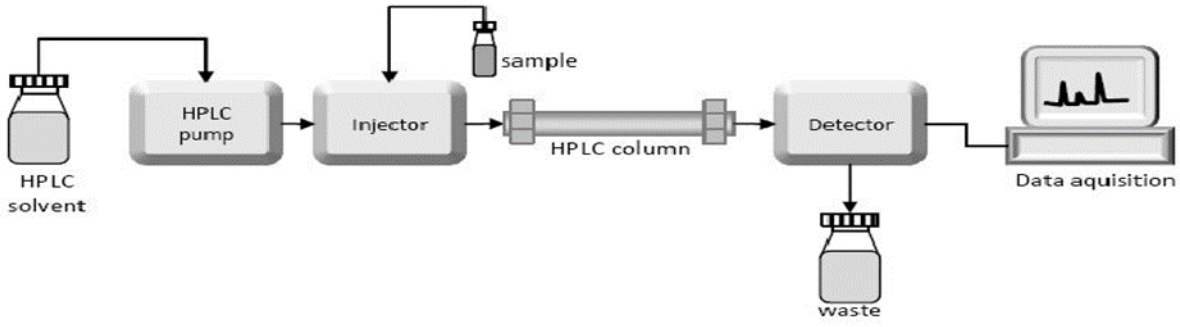
Introduction:

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drugs. Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Dosage forms of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias. Numerous methods have been reported for the analysis of paracetamol and its combinations in pharmaceuticals or in biological fluids. Paracetamol has been determined in combination with other drugs using titrimetry, voltammetry, fluorimetry, colorimetry, UV-spectrophotometry, quantitative thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC)and gas chromatography (GC)in pharmaceutical preparations.

Structure of paracetamol tablet



Flow scheme for HPLC



High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

Sample retention time will vary depending on the interaction between the stationary phase, the molecules being analyzed, and the solvent, or solvents used. As the sample passes through the column, it interacts between the two phases at different rate, primarily due to different polarities in the analytes. Analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

Glossary of HPLC Terms

- HPLC – separation technique for components of organic mixtures involving retention of components on stationary phase packed inside column on the basis of physico – chemical interactions followed by sequential elution
- Stationary phase – solid bed inside column whose particles are coated with the retention phase.
- Mobile phase – liquid carrier medium used for transporting the sample through the HPLC system,
- Normal phase separation – separation mode in which the retention material is polar and mobile phase is nonpolar. Retained sample components are eluted in ascending order of polarity.
- Reverse phase separation – separation mode in which the stationary phase is nonpolar and mobile phase is polar. Elution order of components is in decreasing order of polarity. It is the most commonly used mode of HPLC separations.
- Isocratic Elution – mobile phase composition does not change during the chromatographic run.
- Gradient Elution – mobile phase composition varies during the run and can be programmed before starting the chromatographic run
- Retention time – time taken between injection and the maximum of the peak response of a component

Chemicals:

- Paracetamol
- Methanol: water (3:1)
- Panadol

Procedure

1. Prepare 500 ml from the mobile phase (methanol: water (3:1))
2. Wight 50 mg of paracetamol standard in 100 ml volumetric Flask dissolve it with the mobile phase: methanol: water (3:1) →**stock solution** then sonicate for 5 minutes.
3. Prepare the following standards by using diluent (mobile phase) in 25 ml Volumetric flask from the stock solution using the following table ,

Name	Volume from the stock solution (ml)
Standard 1	2.5
Standard 2	5
Standard 3	7.5
Standard 4	10
Standard 5	12.5
Standard 6	15
Standard 7	17.5

4. Wight Panadol tablet then crush it, take 25 mg from the tablet and dissolve it with the mobile phase in 100 ml volumetric Flask then sonicate for 5 minutes.
5. Measure the absorbance on 276 nm

Experiment 11

Determination of Ethanol in Commercial Products using GC

Objective:

Determination of Ethanol in Bronchicum using GC

Introduction:

Gas chromatography is a chromatography technique that can separate and analyze volatile compounds in gas phase.

All chromatography have one stationary and one mobile phase. In this chromatography, the mobile phase is always gas. However, the stationary phase is either liquid or solid. If the stationary phase is solid, then that is called gas-solid chromatography or GSC. In addition, if the stationary phase is liquid, then that is called gas-liquid chromatography or GLC. In GLC, the mobile gas phase is like helium and the stationary phase is high boiling point liquid adsorbed onto a solid. Like other chromatography, the mobile phase, for this case, is a chemically inert gas, which carry the analyte through the heated column to separate to its individual compounds.

This chromatography consists of an injection port, a column, an oven, a heater to control the temperature, a carrier gas, flow control equipment and a detector.

Procedure

"Performed Method:

Purification and Extraction: Both methanol and ethanol standards must by treated by C18-SPE. Collect 30 ml of methanol and 6 ml of ethanol. Pronchium syrup must be centrifuged for heating purposes in 2x1.5 Eppendorf tubes. Then it is C18-SPE treated. Collect 2 ml of the Pronchium filtrate and keep it for the analysis. GC Conditions: Using TR-WAX column and nitrrogen as the carrier and backup gases start with 3min@45deg. Then raise the temperature to 60deg. at 5deg./min. Hold at 60 for 3 min. Use 1.00 micro as injection volume, 20 as splitting ratio."

Standards

Prepare the following standards

Sample #	$V._{Diluant}$	$V._{EtOH}$	$V._{Prod.}$
1	1	00000	0
2	1	0.0020	0
3	1	0.0040	0
4	1	0.0080	0
5	1	0.0160	0
6	1	0.0200	0
7	1	0.0320	0
8	1	0.0400	0
9	1	0.0500	0
10	1	0.0640	0
11	1	0.0700	0
12	1	0.0800	0
13	1	0.0128	0
14	1	00000	0.07

Measure the concentration of the standard and the sample using GC

Data Sheet

Spectrophotometric Analysis of a Commercial Aspirin Tablet

Name of students in the group:

Weigh of Salicylic acid: ----------

Weigh of Aspirin tablet: ----------

Solution Number	Volume withdrawn from Stock solution of SA (µL)	Final volume of standard solution (mL)	Concentration of standard solutions (M)	Absorbance at 530 nm (A)
1	250	25		
2	500	25		
3	750	25		
4	1000	25		
5	1250	25		

Absorbance of SA in Aspirin tablet ----------

Data Sheet

UV Spectroscopic Analysis of Caffeine and Benzoic Acid in soft drink

Name of students in the group:

Weigh of Benzoic Acid ----------

Concentration of Benzoic Acid in 100 ml ----------

Weigh of Caffeine ----------

Concentration of Caffeine in 100 ml ----------

Benzoic Acid

Concentration (ppm)	Volume of Benzoic Acid (mL)	Final volume of standard solutions (mL)	Absorbance at (230 nm)	Absorbance at (270 nm)
2		25		
4		25		
6		25		
8		25		
10		25		

Caffeine

Concentration (ppm)	Volume of Caffeine (mL)	Final volume of standard solutions (mL)	Absorbance at (230 nm)	Absorbance at (270 nm)
2		25		
4		25		
6		25		
8		25		
10		25		

Absorbance of soft drink at 230 nm ---------- and at 270 nm ----------

Data Sheet

Spectrophotometric Determination of Iron in Vitamin Tablets

Name of students in the group:

Weigh of iron tablet: ----------

Solution Number	Unknown	Hydroquinone	Sodium Citrate	Standard Fe	O-Ph	H_2O	Absorbance at (510 nm)
blank	0	0	15	0	10	Up to 50mL	
1	2.5	5	15	0.5	10		
2	2.5	5	15	1.0	10		
3	2.5	5	15	1.5	10		
4	2.5	5	15	2.0	10		
5	2.5	5	15	2.5	10		

Data Sheet

Spectrophotometric Determination of Paracetamol in Pharmaceutical formulations

Name of students in the group:

Weigh of Panadol tablet: ----------

Maximum absorbance at ---------- nm

Cumulative volume of $Cr_2O_7^{2-}$ added (mL)	Absorbance

Data Sheet

Spectrophotometric Determination of Sulphamethoxazole in Pharmaceutical Formulation

Name of students in the group:

Weigh of Sulphamethoxazole: ----------

Weigh of BalKatrin tablet: ----------

Solution Number	Volume withdrawn from Stock solution of Sulphamethoxazole (µL)	Final volume of standard solution (mL)	Concentration of standard solutions (M)	Intensity of Fluorescence
1	250	25		
2	500	25		
3	750	25		
4	1000	25		
5	1250	25		

Intensity of Fluorescence of Sulphamethoxazole in Balkatrin tablet ----------

Data Sheet

Potentiometric titration of Iron tablet

Name of students in the group:

Weigh of nutrients capsules: ----------

Cumulative volume of Ce added (mL)	Potential (mV)

Cumulative volume of Ce added (mL)	Potential (mV)

Data Sheet

Titration of Phosphoric Acid and Acetic Acid with NaOH

Name of students in the group:

Standardization of NaOH

First trial	
Weigh of KHP	
Volume of NaOH	
Second trial	
Weigh of KHP	
Volume of NaOH	

Acetic Acid

Cumulative volume of NaOH added (mL)	pH

Phosphoric Acid

Cumulative volume of NaOH added (mL)	pH

Data Sheet

Quantitative Analysis of Ascorbic Acid Using Voltammetry

Name of students in the group:

Weigh of ascorbic acid: ----------

Weigh of unknown (Tang): ----------

Cumulative volume of ascorbic acid added (mL)	Concentration of ascorbic acid (mg/L)	Current (A)