Analytical Chemistry Lab (Special Topics)

Selected Experiments

Prepared by

Prof. Dr. Nizam M. El-Ashgar

Professor of Analytical Chemistry

Gaza-Palestine

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Course Overview

This course is designed for chemistry department students. Its aim is to develop competence in areas of chemical analysis of different samples including food products, drugs, alloys and others. Different standard analytical reported methods in addition to instrumental methods are included. Assignments for this course include hands-on experiments with a formal report and quizzes.

After finishing the course students should be able to request appropriate chemical analysis, understand procedures and principles of chemical testing methods, perform laboratory operations and report procedures and results in concise written reports. Students will familiarize with both classical and instrumental methods usually employed in analytical chemistry. Further considerations include the quality of the analytical results and the generation of precise and accurate analytical data. Students must come to lab prepared to make optimal use of the lab period. Each student will maintain his or her own notebook in the style that described bellow and will submit the notebook for grading by the end of the period on the designated dates. In addition to the notebook write-up each student will submit a formal report in the style described below.

Students are required to do all of the assigned experiments. If you are not able to attend a scheduled lab section, make an arrangement in advance by writing to the instructor to see if there is a possibility to get permission to attend another lab in the same week. Only those with a valid written excuse for missing a lab will be considered. Late arrival or the making of phone calls during the lab is not allowed.
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CHEMISTRY LABORATORY RULES AND SAFETY PRECAUTIONS

1- Never work alone in the laboratory.
2- Smoking is not permitted in the building.
3- Unauthorized experiments are prohibited.
4- Know the location and use of the fire extinguisher, safety showers and first aid kit.
5- It is required that you wear prescription glasses or safety glasses at all times in the laboratory for your own protection. Contact lenses are particularly dangerous and they must not be worn in the laboratory.
6- Report all injuries to your instructor at once.
7- Never taste chemicals or solutions.
8- Use the fume hoods for all poisonous reactions or any reactions which produce noxious gases.
9- When diluting concentrated acid or base always add the concentrated acid or base to water (never the reverse), while stirring the solution. Be very careful with sulfuric acid.
10- Keep an orderly, clean laboratory desk. Return glassware to the lab drawer when finished using it to keep the work area from becoming cluttered.
11- Place unneeded books, etc. on the shelves at the side of the laboratory.
12- Waste containers are provided for the disposal of all solid chemicals and paper, etc.
13- Stock reagent bottles are placed on the side bench or beside the balances; leave them at that position.

14- Always read the label **twice** before taking any chemical from a bottle. If you are not sure if you have the right chemical, **ask!**

15- When pouring reagents, hold the bottle so the label points upwards facing the palm of the hand. The accumulation of reagent on bottle lip may be removed by touching the bottle lip to the rim of the receiving vessel.

16- **Avoid** using an **excess** of reagent. If you happen to have measured out too much, see if someone else can use the excess.

17- Due to possible contamination of the contents of a whole stock bottle, never return unused chemical to the stock bottle.

18- Always check your glassware before you use it. If it is broken or cracked, exchange it for a new one.

19- There is one Container reserved for broken glass. All broken glassware should be placed in this crock and no other.

20- If corrosive chemicals or liquids come in contact with the skin or clothing, flood with copious amounts of water for an extended period of time.

21- Spilled chemicals should be wiped up immediately; spilled acid or base should be rinsed with plenty of water and wiped up with a sponge and the sponge rinsed after.

22- Inserting glass tubing or thermometers through a rubber stopper - first lubricate the tube and stopper with glycerol or water, then holding the tube near the end to be inserted insert slowly while rotating the tube. **BE VERY CAREFUL!**

23- When you are ready to leave the laboratory, your bench area should be rinsed off with a wet sponge and the water, gas, and air valves shut off.

24- The chemistry store room is out of bounds to students. If you require apparatus, ask your instructor for it.
Agreement

The previous safety rules must be understood before any laboratory work is begun.

I have read and understood the safety rules that appear on preceding pages of this manual, recognize that it is my responsibility to observe them, and agree to abide by them throughout this course.

Name and number ________________________________

Date______________ Signature ______________________________
Laboratory Notebook Guidelines

The purpose of the laboratory notebook is to provide a written record of all actions performed that contributed to a conclusion based upon experimentation. A professional chemist is legally responsible to assure the accuracy and intent of the contents of this record. For example, an academic or industrial chemist may be involved in patent applications and/or disputes and be required to explain, verify and defend the contents of his laboratory notebook during application or litigation procedures.

In this lab you are required to maintain your notebook as if you were a professional chemist.

- Each page must be numbered sequentially **BEFORE** you begin using it.
- All entries must be made in **ink** (this is a permanent record).
- If you make a mistake or wish to change something **do not erase it, or use white-out** but simply draw a straight line through it. (You never know when you might want to retrieve the data).
- The bottom of each page should be initialed and dated by you as it is completed.
- If you ever have an empty page or section of a page simply put a line through it with your initials.
- It is realized that your notebook is similar to "art in progress". It should accompany you through the various procedures you will be performing in the lab. Even so, you **MUST** make an effort to have **LEGIBLE** entries, even as you are writing "on the fly".
- If you need to insert data taken at a later date (as often happens) or need to rewrite calculations, simply insert a message such as "see page xx for this measurement or calculation."
**NEVER, EVER write data on scrap paper and then later enter it in your notebook.** Invariably the paper will be lost and so with it your data.

- In short, prepare your notebook so that any other person can pick it up, read it and follow it as if it were an abbreviated lab manual.
- Keep in mind that someday it may be more than just a grade you'll have to defend.

**Your notebook must contain the following sections for each experiment:**

1. The **Title** and **Date** the experiment was begun.
2. The **PURPOSE** of the experiment must be stated.
3. The **PROCEDURE** must be written to describe the actual steps performed.
   Do not reiterate the procedure from the lab manual. This should be written in a subjective (nonpersonal) manner and in the **past** tense. Include descriptions and settings or parameters of any instrumentation used including the manufacturers name and model number. A diagram of the instrument used should be included.
4. The **EXPERIMENTAL RESULTS** section must include all measurements made during and for the experiment.
   List all sample masses, volumes, and observations. Include raw data. Graphs should be inserted when necessary. In this section you are to include sample calculations and a summary data table. Any **calculations** used, including statistical analysis must be shown. One example of each type of calculation is sufficient. Results must be shown with the proper sig figs. and units. If multiple measurements are taken, the proper statistical analysis should be included. Any observations or comments should be written in this section.
5. The **DISCUSSION SECTION** will include interpretations of the experimental data. It must include a concluding statement summarizing the results of the experiment.

6. **REFERENCES:** include any citations to information used in the report.
REPORT WRITING
A formal report is required for each one of the experiments. Reports are due at the beginning of the next lab period after you finish an experiment. **There will be a 15% grade deduction on all late submissions** and no report will be accepted after two weeks of the experiment completion have passed.

- Graphs should be plotted on the same size paper and should be scaled so that the data occupies the majority of the plotting area.
- All graphs in the final report, with the exception of your raw data, must be computer generated.
- All axes should be labeled and the proper units displayed. When logarithmic scales are used please make sure the data is accurately represented in your plot.
- When reporting tables of data please check your significant figures, they are important.

**Your final report must consist of the following sections:**

I) **Title Page:**
Title of the experiment, course name and number, your name and the date.

II) **Introduction:**
Presents a description of the parameters to be measured and the general approach to the problem. Should also include a detailed description of the analytical technique involved showing a thorough knowledge of the concepts involved. This section should also state the objectives of the experiment.

III) **Experimental Section:**

1. **Analytical procedure:** This section should include a description of how the experiment was conducted and the equipment was used. Do not copy the procedure section from the lab manual. Write in your own words to
describe how you performed the experiment. Be sure to include any modifications or deviations from the suggested protocols.

2. Raw Data: Record all experimental data as it is obtained. Include the original data sheet with your report (remember an initialized copy of the data has been submitted to the instructor).

IV) Results:

Present your data in tables and graphs. Calculations and error analysis must be shown and explained. Use SI units only. If difficulties were encountered include a narrative description of the problem. All graphs, tables and sections must have a title/caption and should be referenced in your text.

V) Discussion.

Discuss your findings; make comparisons with known values if available. Elaborate on possible sources of errors, selectivity and sensitivity of the technique, detection limits, matrix effects, interferences, accuracy, precision, applicability, etc.

VI) Conclusion:

Present your summary conclusion and suggest any possible improvements in the experiment.

VII. References:

Include references using the IUG Journal of Natural and Engineering Studies journal format:

Example:

For more details, please visit the journal guidelines: http://research.iugaza.edu.ps/Portals/155/Submission%20Guidelines%20for%20IUGNES.pdf
# Checklist for Lab Report Grades

<table>
<thead>
<tr>
<th>Section</th>
<th>Max. Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cover page to include title, names, and date</td>
<td>1</td>
</tr>
<tr>
<td>2 Introduction: Description of technique/Principles/Equations/Reactions</td>
<td>1.5</td>
</tr>
<tr>
<td>3 Objectives: The aim of the experiment</td>
<td>0.5</td>
</tr>
<tr>
<td>4 Experimental Section: Analytical procedure and raw data</td>
<td>1.5</td>
</tr>
<tr>
<td>5 Results: Tables of data/observations/Calculations Graphs fits/equations/error/axes/Units/SD's Analytical Result</td>
<td>3</td>
</tr>
<tr>
<td>6 Discussion/Questions/Definitions: Discuss your results and compare your results with standard values</td>
<td>1.5</td>
</tr>
<tr>
<td>7 Conclusion: Conclude and summaries your findings.</td>
<td>0.5</td>
</tr>
<tr>
<td>8 References.</td>
<td>0.5</td>
</tr>
<tr>
<td>Total grade</td>
<td>10</td>
</tr>
</tbody>
</table>

*Note: If you miss a section you WILL NOT GET THE POINTS.*
Experiment # 1

Determination of Vitamin C Concentration by Titration

Redox Titration Using Iodine Solution

Vitamin C (ascorbic acid) is an antioxidant that is essential for human nutrition. Vitamin C deficiency can lead to a disease called scurvy, which is characterized by abnormalities in the bones and teeth. Many fruits and vegetables contain vitamin C, but cooking destroys the vitamin, so raw citrus fruits and their juices are the main source of ascorbic acid for most people.

One way to determine the amount of vitamin C in food is to use a redox titration. The redox reaction is better than an acid-base titration since there are additional acids in a juice, but few of them interfere with the oxidation of ascorbic acid by iodine. Iodine is relatively insoluble, but this can be improved by complexing the iodine with iodide to form triiodide:

\[ I_2 + I^- \leftrightarrow I_3^- \]

Triiodide oxidizes vitamin C to form dehydroascorbic acid:

\[ C_6H_8O_6 + I_3^- + H_2O \rightarrow C_6H_6O_6 + 3I^- + 2H^+ \]

As long as vitamin C is present in the solution, the triiodide is converted to the iodide ion very quickly. However, when the all the vitamin C is oxidized, iodine and triiodide will be present, which react with starch to form a blue-black complex. The blue-black color is the endpoint of the titration. This titration procedure is appropriate for testing the amount of vitamin C in vitamin C tablets, juices, and fresh, frozen, or packaged fruits and vegetables. The titration can be performed using just iodine solution and not iodate, but the iodate solution is more stable and gives a more accurate result.
Purpose.

The goal of this laboratory exercise is to determine the amount of vitamin C in samples, such as fruit juice.

Procedure.

Equipment Needed
- burette and stand
- 100 mL volumetric flask
- 20 mL pipette
- 250 mL conical flask
- 10 mL and 100 mL measuring cylinders

Solutions Needed.

*Potassium iodate solution: (0.002 mol L$^{-1}$).*
If possible, dry 1 g of potassium iodate for several hours or overnight at 100°C. Allow to cool and accurately weigh about 0.43g of potassium iodate and dissolve in 1 L of distilled water in a volumetric flask.

*Starch indicator solution: (0.5%).*
Weigh 0.25 g of soluble starch and add it to 50 mL of near boiling water in a 100 mL conical flask. Stir to dissolve and cool before using.

*Potassium iodide solution: (0.6 mol L$^{-1}$)*
Dissolve 10 g solid KI in about 50 mL of distilled water in a 100 mL volumetric flask and dilute to 100 mL with distilled water.

*Dilute hydrochloric acid:(1 mol L$^{-1}$).*

Sample Preparation

*For vitamin C tablets:*
Dissolve 1/2 tablet in 100 mL of distilled water (in a volumetric flask if possible).

*For fresh fruit juice:*
Strain the juice through cheesecloth to remove seeds and pulp which may block pipettes.
**For packaged fruit juice:**
This may also need to be strained through cheesecloth if it contains a lot of pulp or seeds.

**For fruits and vegetables:**
Cut a 100 g sample into small pieces and grind in a mortar and pestle. Add 10 mL portions of distilled water several times while grinding the sample, each time decanting off the liquid extract into a 100 mL volumetric flask. Finally, strain the ground fruit/vegetable pulp through cheesecloth, rinsing the pulp with a few 10 mL portions of water and collecting all filtrate and washings in the volumetric flask. Make the extracted solution up to 100 mL with distilled water.
Alternatively, the 100 g sample of fruit or vegetable may be blended in a food processor together with about 50 mL of distilled water. After blending, strain the pulp through cheesecloth, washing it with a few 10mL portions of distilled water, and make the extracted solution up to 100 mL in a volumetric flask.

**Titration**
1- Pipette 5 mL of the sample solution into a 250 mL conical flask and add about 30 mL of distilled water, 2 mL of 0.6 mol L⁻¹ potassium iodide, 2 mL of 1mol L⁻¹ hydrochloric acid and 1 mL of starch indicator solution.
2- Titrate the sample with the 0.002 mol L⁻¹ potassium iodate solution. The endpoint of the titration is the first permanent trace of a dark blue-black colour due to the starch-iodine complex.
3- Repeat the titration with further aliquots of sample solution until you obtain concordant results (titres agreeing within 0.1 mL).

**Titrating Real Lemon**
Real Lemon is nice to use because the maker lists vitamin C, so you can compare your value with the packaged value. You can use another
packaged lemon or lime juice, provided the amount of vitamin C is listed on the packaging. Keep in mind, the amount can change (diminish) once the container has been opened or after it has been stored for a long time.

1. Add 10.00 mL of Real Lemon into a 125 mL Erlenmeyer flask.
2. Titrate until you have at least three measurements that agree within 0.1 mL of iodine solution.

**Result Calculations**

1. Calculate the average volume of iodate solution used from your concordant titres.
2. Calculate the moles of iodate that reacted forming iodine.
3. Using the equation of the reaction between the iodate ions and iodide ions (below) calculate the moles of iodine formed.

\[
2\text{IO}_3^- + 10\text{I}^- + 12\text{H}^+ \rightarrow 6\text{I}_2 + 6\text{H}_2\text{O}
\]

4. From the titration equation (below) determine the moles of ascorbic acid reacting.

\[
\text{ascorbic acid} + \text{I}_2 \rightarrow 2\text{I}^- + \text{dehydroascorbic acid}
\]

5. Calculate the concentration in mol L\(^{-1}\), of ascorbic acid in the solution obtained from fruit/vegetable/juice.

Also, calculate the concentration in mg/100mL or mg/100g of ascorbic acid in the sample.

Listed below are a few juices and their ranges of Vitamin C content.

<table>
<thead>
<tr>
<th>Juice</th>
<th>Vitamin C Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>20–80 mg/100 mL</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>35–65 mg/100 mL</td>
</tr>
<tr>
<td>Lemon</td>
<td>30–70 mg/100 mL</td>
</tr>
<tr>
<td>Lime</td>
<td>5–40 mg/100 mL</td>
</tr>
</tbody>
</table>

For more details visit:
http://www.naturalhub.com/natural_food_guide_fruit_vitamin_c.htm
Methods For Determination of Titratable Acidity in Milk Powder and Similar Products (ROUTINE METHOD)

Scope:
This International Standard specifies a routine method for the determination of the titratable acidity of all types of dried milk.

Definition:
Titratable acidity of dried milk: The number of millilitres of 0.1 N sodium hydroxide solution required to neutralize, in the presence of phenolphthalein, a quantity of the reconstituted milk sample corresponding to 10 g of fat free dry milk solids, until the appearance of a pink coloration.

Principle:
- Preparation of reconstituted milk by addition of water to a test portion of dried milk corresponding accurately to 5 g of fat free dry milk solids.
- Titration with 0.1 N sodium hydroxide solution using phenolphthalein as indicator and cobalt(II)sulphate as reference color solution.
- Multiplication of the number of milliliters used in the titration by the factor 2, in order to obtain the number of milliliters in terms of 10 g of fat free dry milk solids.
- The amount of sodium hydroxide solution required is a function of the amount of natural buffering substances present in the product, and of developed or added acid or alkaline substances.

Reagents:
All reagents shall be of recognized analytical quality. Water shall be distilled or deionized water, free from carbon dioxide by boiling for 10 min before use.

1- Sodium hydroxide standard solution, 0.1 N ± 0.0002.

2- Reference color solution:
Dissolve 3 g of cobalt(II) sulphate heptahydrate (CoSO_4 \cdot 7H_2O) in distilled water and make up to 100 mL.

3- Phenolphthalein solution:
Dissolve 2 g of phenolphthalein in 75 mL of 95 % (v/v) ethanol and make up to 100 mL of water.

**Apparatus and glassware:**
- Analytical balance 0.01 g or better sensitivity.
- Burette, graduated in 0.1 mL and with an accuracy of 0.05 mL.
- Pipettes of capacity 2 mL.
- Measuring cylinders, of capacity 50 mL.
- Conical flasks, of capacity 100 or 150 mL, with ground necks and ground glass stoppers.

**Procedure:**

1) **Preparation of the test sample.**
Transfer the sample to a clean, dry container (provided with an air-tight lid) of capacity about twice the volume of the sample. Close the container immediately, and thoroughly mix the container. During these operations, exposure of the sample to the atmosphere should be avoided as far as possible, to minimize adsorption of water.

2) **Test portion**
Weigh (500 g/a) ± 0.01 g of the test sample into each of two conical flasks.

a: being the fat free dry milk solids content of the sample, expressed as percentage to two decimal places.
NOTE — The fat free dry milk solids content of the sample may be calculated by subtracting the fat content (determined in different exp.) and the moisture content from 100.

3) Determination

- Prepare reconstituted milk by adding 50 mL of water at about 20°C to the test portion and agitating vigorously. Allow to stand for about 20 min.
- Add to one of the conical flasks 2 mL of the reference color solution to obtain a color standard and mix by slight swirling.
- If a series of determination on similar products is to be carried out, this color standard may be used throughout. However it should be discarded after 2 h.
- Add 2 mL of phenolphthalein solution to the second conical flask and mix by slight swirling.
- Titrate the contents of the second conical flask while swirling, by adding the sodium hydroxide solution from the burette until a faint color similar to that of the color standard persist for about 5 s. The titration should be completed within 45 s.
- Record the volume, in milliliters, of sodium hydroxide solution used, to the nearest 0.05 mL.

Expression of results:

Method of calculation and formula

\[2 \times V\]

Where V is the volume, in milliliters, of the 0.1N sodium hydroxide solution used for titration.

Express the result to one decimal place.
Repeatability:
The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0.4 mL of 0.1 mol/L sodium hydroxide solution per 10 g of fat free dry milk solids.

Test report
- The test report shall show the method used and the result obtained.
- It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.
- The test report shall include all details required for the complete identification of the sample.

Percentage titratable acidity:
For calculating the titratable acidity in terms of percentage, the following formula may be used:

Percentage titratable acidity (as Lactic Acid) = 0.09 x 2 x V

Where V= volume in milliliters of sodium hydroxide solution used for titration.
# SPECIFICATIONS CONCERNING RAW MATERIALS FOR RECOMBINING MILK

## Skimmed milk powder

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture level (maximum)</td>
<td>4%</td>
</tr>
<tr>
<td>Fat level (maximum)</td>
<td>1.25%</td>
</tr>
<tr>
<td>Maximum titratable acidity (expressed as lactic acid)</td>
<td>0.10–0.15%</td>
</tr>
</tbody>
</table>

## Whole milk powder

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (maximum)</td>
<td>3.0 %</td>
</tr>
<tr>
<td>Titratable acidity (expressed as lactic acid according to ADMI method) (maximum)</td>
<td>0.15 %</td>
</tr>
</tbody>
</table>

## Buttermilk powder

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content, maximum</td>
<td>4.0 %</td>
</tr>
<tr>
<td>Fat content, minimum</td>
<td>4.5 %</td>
</tr>
<tr>
<td>Titratable acidity (expressed as lactic acid according to ADMI method): limits</td>
<td>0.8–1.6 %</td>
</tr>
</tbody>
</table>
Experiment # 3

Determination of Fruit Acids by Titration and Calculation of the Sugars/Acid Ratio

It is the sugar/acid ratio which contributes towards giving many fruits their characteristic flavor and so is an indicator of commercial and organoleptic ripeness. At the beginning of the ripening process the sugar/acid ratio is low, because of low sugar content and high fruit acid content, this makes the fruit taste sour. During the ripening process the fruit acids are degraded, the sugar content increases and the sugar/acid ratio achieves a higher value. Overripe fruits have very low levels of fruit acid and therefore lack characteristic flavor.

Titration is a chemical process used in ascertaining the amount of constituent substance in a sample, e.g. acids, by using a standard counter-active reagent, e.g. an alkali (NaOH). Once the acid level in a sample has been determined it can be used to find the ratio of sugar to acid.

There are two methods specified for the determination of the titratable acidity of fruits:

- Method using a coloured indicator.

- Potentiometric method, using a pH meter, which should be used for very coloured juices.

Material:

- A laboratory burette of 25 or 50mL capacity or an automatic burette is used. A 10mL pipette, beaker (250mL), a filter (muslin cloth or fine filter) and an extractor or homogenizer.

- A bottle of distilled water.
- **Sodium Hydroxide (NaOH):**

The Standard Laboratory solution of 0.1M which is used in the actual titration is considered to be dilute, and can readily be purchased in this form.

- **Phenolphthalein:**

This is a 1% w/v solution of phenolphthalein in 95% v/v ethanol which is flammable and toxic if ingested. This is only required for the method using a coloured indicator.

- **Indicator stripes:**

To check the exact point of neutrality an indicator stripe should be used. Not necessary if pH Meter is used.

**Sampling**

To evaluate the lot selected for inspection, take a sample of at least 10 fruits of each size at random from the reduced sample. However, fruits should be free from defects such as sun scorch and pest or disease damage, which may have affected the normal ripening process.

**Sample preparation**

Depending upon the type of produce, either cut the fruit in half and squeeze out the juice with an extractor or a juice-press e.g. citrus fruits, or homogenize the flesh into a pulp. The juice of all squeezed fruits is mixed.

The skin and solids should not be included; the solids being filtered out through muslin cloth or fine filter extracting as much juice as possible.

Use a clean and dry safety 10mL pipette. Draw up 10mL of juice and discharge it into a 250mL beaker. Using another clean and dry pipette draw up 50mL of distilled water and add to the juice in the beaker.
**Measurement**

**Method using a colored indicator.**

Add 3 drops of phenolphthalein to the juice/water solution in each beaker from a dropping pipette which is specifically kept for that purpose.

Ensure the tap on the burette is shut and using a funnel pour the 0.1M solution of NaOH into the burette until it reaches the zero mark. Do not spill the solution onto the skin.

Slowly titrate the NaOH into the juice/water solution (with a 25mL burette or an automatic burette). Care must be taken that the NaOH is dropped directly into the solution and does not adhere to the glass, otherwise the reading may be false. While titrating care must be taken to continually swirl the solution in the beaker to keep it thoroughly mixed. This is essential, particularly when the solution nears neutrality. It is important to determine the point of neutrality or the end point of titration very exactly. The phenolphthalein indicator changes very rapidly from colorless to pink and the end point can easily be missed, which will give an inaccurate reading for the test. It is important therefore that towards the end of the titration the NaOH is added a drop at a time. Using phenolphthalein as an indicator, the point of neutrality is reached when the indicator changes from colorless to pink. The indicator color must remain stable (persisting for 30 seconds) and be light pink when viewed over a white background. However, the shade can vary depending on the type of juice being tested. If the point of neutrality is missed, i.e. the color of the indicator is too dark, the test is not acceptable and must be repeated. An indicator stripe should be used to avoid the neutral point of pH 8.1.

- Read off the amount of NaOH used (titre) on the burette and record this figure.
- Re-fill the burette for each subsequent test.
- Clean the equipment thoroughly and rinse with distilled water. Detergents must not be used.

*Note: When testing very acidic juices e.g. lemons and limes a larger amount of NaOH is required.*

Therefore, when the NaOH reaches the 25mL mark on the scale the burette tube should be recharged as described above. When the end point is reached the various readings are added together and recorded to produce a figure of NaOH used for each titration.

**Method using a pH meter.**

The point of neutrality i.e. the end point of titration may also be determined using a pH meter. The precise method used will depend on the manufacturer instructions, but the following will provide a general guide.

**Checking the pH meter**

- Make sure the pH meter has warmed up before use - allow about 30 minutes.
- Remove the electrode from the distilled water in the storage beaker and dry.
- Place the electrode into the beaker containing a buffer solution of pH 7 and calibrate the meter to the same figure.
- Whenever readings are taken, ensure that the electrode is not in contact with the sides or base of the beaker.
- Remove the electrode and - after rinsing in distilled water - place in the solution to be tested; the electrode should not have any contact with the glass.

**Measurement**

Ensure the tap on the burette is shut and using a funnel pour the 0.1M solution of NaOH into the burette until it reaches the zero mark. Do not spill the solution onto the skin.
Slowly titrate the NaOH into the juice/water solution. Care must be taken that the NaOH is dropped directly into the solution and does not adhere to the glass, otherwise the reading may be false.

While titrating care must be taken to continually swirl the solution in the beaker to keep it thoroughly mixed. This is essential, particularly when the solution nears neutrality. It is important to determine the point of neutrality or the end point of titration very exactly. The end point can easily be missed, which will give an inaccurate reading for the test. It is important therefore that towards the end of the titration the NaOH is added a drop at a time.

Using a pH meter, while titrating the digital readout will be seen to climb from around 4 or 5.

When the reading reaches 7 proceed carefully. The point of neutrality or the end point of titration is reached at pH 8.1. If this figure is exceeded the test is not acceptable and must be repeated.

- When the pH meter reads 8.1 read off the amount of NaOH used on the burette and record.
- Remove the electrode and rinse it in distilled water ready for the next test. Do not allow it to become contaminated.
- Re-fill the burette for each subsequent test.
- Clean the equipment thoroughly and rinse with distilled water. Detergents must not be used.

Note: When testing very acidic juices e.g. lemons and limes a larger amount of NaOH is required. Therefore, when the NaOH reaches the 25mL mark on the scale the burette tube should be recharged as described above. When the end point is reached the various readings are added together and recorded to produce a figure of NaOH used for each titration.
**Calculation of the Sugar/Acid Ratio**

The °Brix value of the fruit concerned must also be obtained before calculation of the sugar/acid ratio is possible.

The calculations for determining the sugar/acid ratios of all produce are the same, but as some products contain different acids the appropriate multiplication factor must be applied to each calculation. Some products may contain more than one type of acid, it is the primary acid that is tested. A list of these acids and multiplication factors are found below.

Factor for:
- citric acid : 0.0064 (Citrus fruit)
- malic acid : 0.0067 (Apples)
- tartaric acid : 0.0075 (Grapes)

*Using citric acid as an example, 1mL 0.1M NaOH is equivalent to 0.0064g citric acid (MWt = 192.12) as:*

\[
3 \text{ mol NaOH} \leftrightarrow 1 \text{ mol Citric acid}
\]

Results expressed as percentage acid:

\[
\text{Percentage acid} = \frac{\text{Titre} \times \text{acid factor} \times 100}{10 \text{ (mL juice)}}
\]

\[
\text{The sugar acid ratio} = \frac{\text{Brix value}}{\text{Percentage acid}}
\]

OR

Results expressed as acid in grams/liter

\[
\text{g/L acid} = \frac{\text{Titre} \times \text{acid factor} \times 100 \times 10}{10 \text{ (mL juice)}}
\]

\[
\text{The sugar acid ratio} = \frac{\text{Brix value} \times 10}{\text{g/L acid}}
\]
Results:
It is important to record the results, to one decimal place, as well as all the details concerning method, variety and stage of maturity and ripeness of the produce being tested.
If the result achieves the limit specified in the standard, the lot has reached the minimum maturity level.
If the result is at least 10 per cent below/above the limit specified in the standard, a second sample needs to be taken and analysed with other fruits of the reduced sample or from a new sample.
If the average of the two samples is below/above the limit specified in the standard, the lot fails the minimum maturity level and needs to be rejected. No tolerance is applied.

Health and Safety Guidelines

Sodium Hydroxide in its undiluted form is extremely corrosive to body tissue. Skin contact causes irritation almost immediately and continued contact causes burns. The 0.1 Molar solution used in this test is much safer. However, it is recommended that protective coats are worn when using, and that it is used only in a well ventilated room.

Phenolphthalein is highly flammable and should be used with care. It should be stored and used away from naked flames or other sources of ignition. It is toxic if ingested.

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INDUSTRY STANDARDS

The following shows the industry standards for citrus fruits in some states of Australia. These values can vary in different states.

Table 1. Total soluble solids (°Brix) to acid ratio Fruit Type Total soluble solids (°Brix) to acid ratio

<table>
<thead>
<tr>
<th>Type of Fruit</th>
<th>Acid/Brix ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandarins</td>
<td>8 to 1</td>
</tr>
<tr>
<td>Oranges (navel only)</td>
<td>9 to 1</td>
</tr>
<tr>
<td>Oranges (other than navel)</td>
<td>8 to 1</td>
</tr>
<tr>
<td>Grapefruit and pummelos</td>
<td>5.5 to 1</td>
</tr>
<tr>
<td>Hybrids of any species</td>
<td>8 to 1</td>
</tr>
</tbody>
</table>

Table 2. Total soluble solid determinations (°Brix) Fruit Type Total soluble solids (°Brix)

<table>
<thead>
<tr>
<th>Type of Fruit</th>
<th>°Brix at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandarins</td>
<td>8</td>
</tr>
<tr>
<td>Oranges</td>
<td>8</td>
</tr>
<tr>
<td>Grapefruit and pummelos</td>
<td>8</td>
</tr>
<tr>
<td>Hybrids of any species</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3. Minimum juice content Fruit Type Minimum juice content (%)

<table>
<thead>
<tr>
<th>Type of Fruit</th>
<th>Minimum % juice content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemons</td>
<td>25</td>
</tr>
<tr>
<td>Mandarins</td>
<td>28</td>
</tr>
<tr>
<td>Oranges</td>
<td>33</td>
</tr>
<tr>
<td>Grapefruit and pummelos</td>
<td>33</td>
</tr>
<tr>
<td>Limes</td>
<td>33</td>
</tr>
<tr>
<td>Hybrids of any species</td>
<td>33</td>
</tr>
</tbody>
</table>
Experiment # 4  
**Determination of Salt (Sodium Chloride) in food samples**

Sodium occurs naturally in virtually all foods, albeit in relatively small amounts. Table salt, in the form of sodium chloride (NaCl), is a common additive to food products and is used as a preservative and a flavor enhancer. Traditionally, salt was added to food as a form of preservation. Since the advent of refrigeration, salt is more commonly used to enhance flavor but its ability to reduce microbial growth, improve texture, and increase shelf life are still utilized. Sodium may be added in forms other than table salt, such as sodium nitrate, sodium bicarbonate (baking soda), and monosodium glutamate. Sodium can also be added during food production from more complex sources, such as in soy sauce, garlic salt, or other condiments.

**Effects on Health**

Sodium is an essential nutrient in the human body, but is only needed in relatively small quantities. It plays a critical role in the body’s ability to control blood pressure and blood volume. However, as sodium intake increases, health risks such as high blood pressure increase. Monitoring and maintaining healthy blood pressure levels reduces the risk of cardiovascular disease, congestive heart failure, and kidney disease. The major source of our daily sodium intake is from table salt (sodium chloride). The Institute of Medicine states that for individuals age 9 to 50, the Adequate Intake (AI) level for sodium is 2,300 milligrams per day. The AI levels are recommended daily average intake amounts of a specific nutrient. For infants, whose calorie requirements differ widely from adults, the sodium AI level is significantly lower. Individuals with pre-existing hypertension or other cardiovascular
conditions are also generally advised to limit sodium content below the recommended AI as well.

**Purpose**

This standard testing method is to explain step by step method for determination of Sodium Chloride in a given food or feed sample.

**Scope:**

This determination is generally simpler, easier, and faster method. It is the official A.O.A.C. method for determining of salt content of most foods and is particularly suited for products which are not highly colored so as to interfere with the end point.

**Principle:**

The addition of standard AgNO₃ to a sample solution, using K₂CrO₄ as the visual indicator, yields an insoluble precipitate which is proportional to the amount of total chlorides in the solution.

The red-colored silver chromate complex, formed by the combination of AgNO₃ and K₂CrO₄, is soluble in acid and loses its color. The salt content of the sample may be calculated from the volume of standard AgNO₃ used to reach the end point. The chemical reaction is:

\[
\text{AgNO}_3 + \text{NaC1} \rightarrow \text{NaNO}_3 + \text{AgC1}_\text{(s)}
\]

(white)

When all the AgC1 (silver chloride) has been precipitated, a red-brown color appears which denotes the end point. This color is the result of the formation of a second precipitate (Ag₂CrO₄) as shown by the following equation:

\[
2\text{AgNO}_3 + \text{K}_2\text{CrO}_4 \rightarrow \text{Ag}_2\text{CO}_4 + 2\text{KNO}_3
\]

(red brown)
1. Equipments:
   a) Standard titration burette with stand
   b) Erlenmeyer flask
   c) Pipette
   d) Analytical Balance

2. Reagents:
   e) Distilled Water
   f) 0.1N AgNO₃ (Silver Nitrate)
   g) CaCO₃ (Calcium Carbonate) for acid products.
   h) K₂CrO₄ (Potassium Chromate) indicator

4. Procedure:
   a. Fill the clean burette with 0.1 N AgNO₃. Always read the burette scale with reference to the bottom of the meniscus of the AgNO₃.
   b. Put the prescribed amount of sample into the Erlenmeyer flask.
   c. Dilute with about 25 mL of distilled water. This increases the volume of solution in the flask so that better agitation can be obtained and the end point easier to detect (use boiling distilled water for fatty samples).
   d. Neutralize any naturally occurring acidity, such as in pickles and sauerkraut. Addition of about 0.5 grams of powdered CaCO₃ (calcium carbonate) is usually sufficient and the simplest method of neutralization. This need not be weighed and can be estimated by using the tip of a spatula.
   e. Add approximately 2 mL (4 to 5 drops) of K₂CrO₄ indicator.
f. Titrate with AgNO₃ to the characteristic red-brown end point of the chromate indicator.

5. Calculations.

After determining the amount of AgNO₃ (to the nearest 0.1 mL) necessary to exactly reach the end point, calculate the salt content according to the following formula:

\[
Salt \ Content = \frac{(T)(N)(0.05845) (100)}{V}
\]

Where;

\( T \) = mL of AgNO₃ required to reach the end point

\( N \) = Normality of the AgNO₃

\( V \) = Sample size - either in mL. or grams depending upon the terms in which the results are expressed.

0.05845 = the factor necessary to convert the titration to NaCl and represents the number of grams of NaCl which will completely react with one mL of Normal AgNO₃.

100 = the factor necessary to convert the results into percent by weight or volume.

Precautions:

1) To reduce experimental error, a sample of adequate size should be used so that at least 20 mL of AgNO₃ are required to precipitate all the chlorides.

2) Any naturally occurring acidity must first be neutralized (using CaCO₃); otherwise no visual end point may be attained.
Experiment #5

The Quantitative Determination of Caffeine in Nonalcoholic Beverages

Introduction

Caffeine, a nervous system stimulant, is naturally present in coffee and is incorporated into many non-alcoholic beverages. The stimulant caffeine is incorporated into many nonalcoholic beverages. The method described below for the quantitative determination of caffeine is recommended by the Association of Official Analytical Chemists as an official method. In this experiment, we will compare the amount of caffeine present in Coca-cola and Pepsi. The method described below uses the instrumental technique of UV/Visible spectrophotometry.

Apparatus.

Two Spectrophotometer Cuvet 200-1000 µL pipette
50 mL beaker Six test tubes
50-200 µL pipette 100 mL grad cylinder
Four 100 mL Volumetric Flasks Filter Paper
125 mL vacuum flask 125 mL separator funnel
Two 250 mL beaker magnetic stir bar

Chemicals.

Potassium Permanganate Sodium Hydroxide
Recrystallized Caffeine Chloroform
Sodium Sulphite Phosphoric acid
Potassium Thiocyanate

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A. Reagents
(a) To prepare reducing solution dissolve 5g Na$_2$SO$_3$, and 5 g KSCN in water and dilute to 100 mL.
(b) Dilute phosphoric acid solution - Dilute 15 mL H$_3$PO$_4$ to 85 mL with water.
(c) Sodium hydroxide solution - Dissolve 25 g NaOH in 75 mL of water.
(d) Caffeine standard solutions - Dissolve 50 mg caffeine in CHCl$_3$ and dilute to 50 mL with CHCl$_3$. (concentration = 1mg/mL).

B. Preparation of Standard Curve.

Prepare diluted standard solutions containing 0.10, 0.25, 0.50, 1.00, 1.50, and 2.00 mg caffeine/10 mL CHCl$_3$. Determine the absorbance of all solutions at 276 nm (see Note 1). Prepare a plot of absorbance versus concentration.

C. Determination of Caffeine in Samples
1. Degas the beverage samples by using a vacuum aspirator (Take care not to get the tap water into your sample).
2. Pipette 5 mL degassed sample into a 125 mL separator funnel; add 2.5 mL of 1.5% w/v KMnO$_4$ solution and mix.
3. After exactly 5 minutes, add 5 mL of reducing solution and mix.
4. Add 0.5 mL of the dilute H$_3$PO$_4$ solution, mix, add 0.5 mL of NaOH solution and mix
5. Extract with 25 mL of CHCl$_3$ for 1 minute.
6. After separation, drain the lower layer through 7 cm filter paper into a 50 mL ground stoppered volumetric flask. Add 2-3 mL CHCl$_3$ to the separator and drain through paper to rinse the separator stem.
7. Wash the paper with 2-3 mL of CHCl$_3$. 

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8. Re-extract the solution with 15 mL CHCl₃, and wash stem, and paper as before.
9. Dilute to volume (50 mL) with CHCl₃.
10. Determine the absorbance of this solution at the wavelength of maximum absorbance against CHCl₃ with matched cells.
11. If the absorbance is out of range dilute 1:5 by CHCl₃.
12. Determine the amount of caffeine in your samples from the standard curve.
13. Report the caffeine content as mg caffeine/100 mL of the beverage.

Notes
(1) You will be using only one UV cell for the absorbance determinations. Therefore it will be necessary to obtain a baseline correction run of CHCl₃ against a reference of air.

--------------------------------------------------
Experiment # 6
Simultaneous Determination of Caffeine and Paracetamol in an Analgesic by Ultraviolet Spectrophotometry

Introduction
Whenever possible it is advantageous to analyze a mixture for its components without performing a prior separation of the components. In certain circumstances, spectrophotometry can be used for the simultaneous analysis of various analytes in a mixture. In an ideal case, each component of a mixture exclusively absorbs radiation at a particular wavelength and does not absorb radiation at a wavelength of any other component. In that case, Beer's law can be used at the wavelength which is characteristic of each component to determine its concentration.

Since ultraviolet-visible absorption bands of polyatomic species are usually broad, it is rarely possible to find a wavelength for each component at which no other component absorbs radiation. If a wavelength is chosen for an analysis at which more than one analyte absorbs radiation, then ideally the absorbance of the mixture at that wavelength is the sum of the absorbance of its components. For a sample which contains two absorbing components (analytes), the absorbance at a wavelength $\lambda_1$ is given by the equation:

$$A_{\lambda_1, \text{sample}} = A_{\lambda_1,1} + A_{\lambda_1,2}$$

where $A_{\lambda_1,1}$ is the absorbance at wavelength $\lambda_1$ of component 1 and $A_{\lambda_1,2}$ is the absorbance of component 2. Substitution from Beer's law into the equation yields:

$$A_{\lambda_1, \text{sample}} = \varepsilon_{\lambda_1,1} b C_1 + \varepsilon_{\lambda_1,2} b C_2$$

The values of $\varepsilon_{\lambda_1,1}$, $\varepsilon_{\lambda_1,2}$ and $b$ can be independently measured, and $A_{\lambda_1, \text{sample}}$ is obtained from the absorption measurement. Substitution of these values into the equation results in a single equation with two unknown terms ($C_1$ and $C_2$). Consequently for a two component mixture,
absorbance measurements must also be made at a second wavelength $\lambda_2$ for which the following equation can be written.

$$A_{\lambda_2, \text{sample}} = \varepsilon_{\lambda_2, 1} bC_1 + \varepsilon_{\lambda_2, 2} bC_2$$

The two equations can be simultaneously solved for the concentrations of the two analytes. Although it is theoretically possible to use any two wavelengths for the absorbance measurements, in practice, the accuracy limitations of the measuring instrument make it desirable to choose two wavelengths at which $\varepsilon_1$ and $\varepsilon_2$ significantly differ; i.e., two wavelengths are chosen such that at one wavelength component 1 absorbs strongly and component 2 weakly, and at the other wavelength component 2 absorbs strongly and component 1 weakly.

In addition, the two wavelengths must be chosen such that Beer's law is obeyed and their absorbances are additive.

**Aim of the experiment:**

In this experiment, an analgesic capsule or tablet is simultaneously analyzed for paracetamol (an analgesic) and caffeine (a stimulant) (Fig. 1).

The use of the mixture of paracetamol and caffeine as an analgesic and antipyretic is well established in pharmaceutical formulation. In order to achieve better curative effect and lower toxicity, it is very important to control the content of paracetamol and caffeine in pharmaceutical tablets.

![Paracetamol](image1.png)

**Paracetamol** (Mwt = 151.16)

$\lambda_{\text{max}} = 243$ nm

![Caffeine](image2.png)

**Caffeine** (Mwt = 194.19)

$\lambda_{\text{max}} = 273$ nm
Fig. 1: Overlay spectra of PARA and CA [Ref: Vichare et al. IJPRIF 201]

**Apparatus.**

11, 50mL Volumetric Flask  
Mortar and Pestle  
3, 250mL Volumetric Flask  
10mL Graduated Cylinder  
200-1000 µL  
Eppendorf pipette

**Chemicals.**

Paracetamol  
Caffeine (sublimed)  
Anacin or other analgesic tablet  
Methanol

**Procedure.**

(1) Weigh 0.121 g of caffeine to the nearest 0.1 mg. Quantitatively transfer to a 250 mL volumetric flask using the solvent methanol. Fill the flask to the mark with methanol (conc. caffeine is approx 2.50 x 10^{-3} M).

(2) Prepare 5 different concentrations (25, 50, 75, 100 and 125 µM) of standard solutions using 10 mL volumetric flasks for constructing the calibration curve. Dilute each flask to the mark with methanol.
(3) Weigh 0.094 g of paracetamol to the nearest 0.1 mg. Quantitatively transfer the paracetamol to a labeled 250 mL volumetric flask using methanol as the solvent. Make solution up to the mark with methanol (conc. is approx. 2.50 x 10^{-3} M).

(4) Prepare 5 different concentrations (25, 50, 75, 100 and 125 µM) of standard solutions using 10 mL volumetric flasks for constructing the calibration curve. Dilute each flask to the mark with methanol.

(5) Use Eppendorf pipet to deliver 1.0 mL of the caffeine stock solution and 1.0 mL of the paracetamol stock solution to a 50 mL volumetric flask. Fill the flask to the mark with methanol and mix well.

(6) Weigh to the nearest 0.1 mg the sample analgesic (∼1/4 tablet) provided. Transfer the weighed solid to a labeled 50-mL volumetric flask. Add about 30 mL of methanol to dissolve the sample and then fill the flask to the mark with methanol.

(7) Use an Eppendorf pipet to transfer accurately 0.1 mL of the sample solution into each of three 10 mL test tubes. Dilute each flask to (10 mL) total volume with methanol.

(8) Obtain a baseline spectrum of the methanol solvent referenced against air from a λ_{min} of 220 nm to λ_{max} of 320 nm.

(9) Obtain a baseline corrected spectra, between the same wavelength limits, of the solution prepared in step (5), and of the three sample solutions which were prepared in step (7). Label each spectrum.

(10) Obtain spectra for the five caffeine solutions which were prepared in step (2) over the same limits.

(11) Obtain spectra for the five paracetamol solutions which were prepared in step (4) over the same limits.

**Calculations**

(1) Calculate the concentrations of the caffeine and paracetamol which are in the stock and standard solutions.
(2) From the caffeine spectra choose a wavelength (about 273 nm) on a maximum of the absorbance spectra.

(3) Similarly choose a second wavelength (about 243 nm) from the paracetamol spectra.

(4) Tabulate the absorbance of each of the solutions at the two chosen wavelengths.

(5) Prepare two working curves by plotting the absorbance of each of the standard caffeine solutions as a function of concentration at each of the two wavelengths.

(6) Similarly prepare two working curves for the standard paracetamol solutions.

(7) If the curves are linear and go through the origin, Beer's law is obeyed for each component at each wavelength. If the curves are not linear and/or do not go through the origin, choose a different pair of wavelengths for the analysis. If Beer's law is obeyed use the slope of the working curves to calculate the molar absorptivity of each component at each wavelength.

(8) Use the molar absorptivities to calculate the expected, correct absorbance at each wavelength for the solution which contains a known concentration of both paracetamol and caffeine (prepared in step (5)). If the calculated absorbance agrees with the observed absorbance within 5-10%, the absorbance of the two components are additive at the two chosen wavelengths. If there is disagreement, a new pair of wavelengths must be chosen.

(9) Write an equation of the following form at each of the two wavelengths
\[ A_{\lambda, \text{sample}} = \varepsilon_{\lambda, \text{caff}} bC_{\text{caff}} + \varepsilon_{\lambda, \text{para}} bC_{\text{para}}. \]
Make the proper substitutions into the two equations and solve for \( C_{\text{caffeine}} \) and \( C_{\text{paracetamol}} \) in each of the three sample solutions.

(10) Determine the mean and standard deviation of the percent paracetamol and caffeine in each analgesic tablet.
**Calculations:**

The concentrations of drug contents can be determined using following equations.

\[
C_{\text{para}} = \frac{(A_2 \cdot \varepsilon_{\text{cafe}, \lambda_1} - A_1 \cdot \varepsilon_{\text{cafe}, \lambda_2})}{(\varepsilon_{\text{para}, \lambda_2} \cdot \varepsilon_{\text{cafe}, \lambda_1} - \varepsilon_{\text{para}, \lambda_1} \cdot \varepsilon_{\text{cafe}, \lambda_2})}
\]

\[
C_{\text{caff}} = \frac{(A_1 \cdot \varepsilon_{\text{para}, \lambda_2} - A_2 \cdot \varepsilon_{\text{para}, \lambda_1})}{(\varepsilon_{\text{para}, \lambda_2} \cdot \varepsilon_{\text{cafe}, \lambda_1} - \varepsilon_{\text{para}, \lambda_1} \cdot \varepsilon_{\text{cafe}, \lambda_2})}
\]

*Where*

\[C_{\text{para}} = \text{Concentration of Paracetamol}\]

\[C_{\text{caff}} = \text{Concentration of caffeine}\]

\[A_2 = \text{Absorbance at 273nm}\]

\[A_1 = \text{Absorbance at 243nm}\]

\[\varepsilon_{\text{para}, \lambda_1} = \text{absorptivity of PARA at 243 nm}.\]

\[\varepsilon_{\text{cafe}, \lambda_1} = \text{absorptivity of CAF at 243 nm}.\]

\[\varepsilon_{\text{para}, \lambda_2} = \text{absorptivity of PARA at 273 nm}.\]

\[\varepsilon_{\text{cafe}, \lambda_2} = \text{absorptivity of CAF at 273 nm}.\]
**Experiment # 7**

**The Quantitative Determination of Sodium and Potassium in Fruit Juices by Flame Photometry**

**Introduction:**

The experimental procedure described below for the determination of sodium and potassium has been adapted from an official procedure reported by the Association of Official Analytical Chemists and may be used to obtain the sodium and potassium contents of many fruits and their juices. This method involves the dilution of the fruit juice sample with water followed by filtration and finally aspiration directly into the Flame Photometer.

**Apparatus:**

Seven 50 mL Volumetric Flasks

Glass Funnel

50 mL beaker

100 Erlenmeyer flask

1L Volumetric Flasks

Large beaker for waste

Eight stoppers

Filter Paper

10mL beaker

5mL and 10mL pipette

**Chemicals:**

Dried Sodium Chloride and potassium chloride.

**Preparation of Standard Solutions:**

1- Dry reagent grade sodium chloride and potassium chloride at 100 °C overnight.

2- Allow samples to come to room temperature in a desiccator.
3- Prepare 1 L of 1000 ppm of each Na and K standard solutions using deionized water.

4- Prepare five 50 mL standard solutions of each Na and K of concentrations: 10, 20, 30, 40, and 50 ppm.

**Preparation of Juice samples:**

1- Mix containers of juice thoroughly by shaking. This will ensure uniform sampling.

2- Filter the juices through absorbent cotton or rapid paper and collect the filtrate (take care to remove all solids).

**Flame Photometry Measurements:**

1- Open the valve on the propane regulator.

2- Turning on the power switch to the instrument will automatically ignite the burner.

3- Open the viewing port to ensure that the flame is burning evenly and does not flicker.

4- Aspirate your reference solution from a 10 mL beaker. Zero the digital scale using the "zero" control for sodium (n.b. the instrument scale should be placed in reduced range mode by depressing the Na "Range" button).

5- Replace the reference solution with the most concentrated standard solution and adjust the scale to approximately 50 using the "Calib" control.

6- Aspirate the other standard solutions from 10 mL beakers and record the resultant relative intensities, which if possible should read 300 or as high as is possible.

7- Allow readings to stabilize before recording.

8- Remember to re-zero the instrument with the reference solution between each reading and repeat each measurement twice.
9- Construct a working curve of relative intensity versus concentration from your results.

10- Note: If the Na and K concentrations in the fruit juice are outside the range of standards the sample should be diluted accordingly.

**Calculations:**

1- Use the working curve constructed from your standard solutions, the relative intensities of your juice samples and any relevant dilution factors to calculate the mean, and standard deviation of ppm sodium and potassium in each juice sample.

2- Compare your results with the recommended healthy or world standard values.

------------------------------------------------------
Experiment # 8

Honey Analysis

A) DETERMINATION OF MOISTURE, REFRACTOMETRIC METHOD

SCOPE:
The standard describes a procedure to measure the water content of honey.

DEFINITION
The water content is that value determined from the refractive index of the honey by reference to a standard table.

Importance:
Honey moisture is the quality criterion that determines the capability of honey to remain stable and to resist spoilage by yeast fermentation: the higher the moisture, the higher the probability that honey will ferment upon storage. The determination of moisture by refractometry does not yield the true water content and yields lower values than the Carl Fischer method. However, it is a very simple and reproducible method, successfully used up to the present time and thus there is no need for alternative methods. The VKR values varied from 0.8 to 2% over the whole determination range.
Lower moisture limits (e.g. 19%), ensuring a better shelf-life of honey which would be met by a large majority of the commercial honeys, have been proposed by some countries for the revision of the Codex Alimentarius.

PRINCIPLE
The method is based on the principle that refractive index increases with solids content.
The table was constructed from a plot of the logarithm of the refractive index minus unity plotted against the water content as determined by vacuum drying, a technique which requires much greater manipulative skill.

**EQUIPMENT.**

Flasks, 50 mL  
Water bath.

Abbé or a digital refractometer, that can be thermostated at 200°C, regularly calibrated with distilled water or with another certified reference material.

The refractive index for water (nD) at 20°C is 1.3330.

**PROCEDURE**

*Sample preparation.*

**Liquid or crystallised honey free from extraneous matter.**

Homogenize the laboratory sample by stirring thoroughly (at least three minutes). Be careful that as little air as possible is stirred into the honey.

**Liquid or crystallized honey containing extraneous matter.**

Remove any coarse material, subsequently stir the honey at room temperature and pass through a 0.5mm sieve. Gently press crystallized honey with a spatula through a 0.5mm sieve.

**Comb honey.**

Uncap the comb. Drain the comb through a 0.5mm sieve without heating in order to separate honey from the comb.

**Dissolution**

- Homogenize the prepared sample again and put in a flask. Close the flask and place in a water bath at 50°C (±0.2) until all the sugar crystals are dissolved.
- Cool the solution to room temperature and stir again.

*Note: Ensure that the flask is air tight.*

**Determination**
- Ensure that the prism of the refractometer is clean and dry.
- Directly after homogenisation, cover the surface of the prism evenly with the sample.
- After 2 minutes (Abbe refractometer) read the refractive index.
- Measure each honey twice and take the average value.
- Read the corresponding moisture content from the table.
- Carefully clean the prism after use.
- \textit{Note:- The method refers only to the use of the Abbé refractometer, not to digital instruments, which appear to require an elapsed time of four minutes before reading.}
- Reproducibility data for the digital instrument is not available.
**RELATIONSHIP OF WATER CONTENT OF HONEY TO REFRACTIVE INDEX:**

<table>
<thead>
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<th>Water Content g/100 g</th>
<th>Refractive Index 20°C</th>
<th>Water Content g/100 g</th>
<th>Refractive Index 20°C</th>
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<td>1.4740</td>
</tr>
</tbody>
</table>

Temperatures above 20° C: add 0.00023 per °C.

Temperatures below 20° C: subtract 0.00023 per °C.
B) Determination of Sugars in Honey by Lane-Eynon method

Introduction:
Honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. The definition of honey stipulates a pure product that does not allow for the addition of any other substance. This includes, but is not limited to, water or other sweeteners.

The Lane-Eynon method is an example of a titration method to determine the concentration of reducing sugars in a sample. Reducing sugars are those which, in an alkaline solution, form an aldehyde or ketone allowing the sugar to act as a reducing agent. In the Lane-Eynon method copper ions are reduced to copper oxide. Although fructose is not considered a reducing sugar in its own right it is converted to glucose and mannose under alkaline conditions. Sucrose is not detected since the anomeric (reducing) carbon of glucose is involved in the glucose- fructose bond is not free to form the aldehyde in solution5. This method was used to determine the total reducing sugars content of honey samples and could be modified to determine the Total sugars and sucrose by difference.

Reagents and apparatus:
1) Fehling’s solutions A and B
2) Honey samples
3) Glucose standard solution (0.5%)
4) 0.2% methylene blue indicator

Procedure:
2) Reducing Sugars.
1- A 50 mL burette was filled with an accurately prepared standard glucose (0.5%) solution.
2- Fehling’s solutions A and B, 50 mL of each, were mixed in a beaker. It was calculated that this would be sufficient for the entire analysis to avoid standardization of a fresh batch.
3- A 25mL aliquot of mixed Fehling’s was pipetted into a 150 mL conical flask and 22 mL glucose solution (0.5%) from the burette was added.
4- The mixture was heated to boiling whilst stirring with a magnetic stirrer and maintained at boiling for 2 minutes.
5- Methylene-blue indicator solution (2-3 drops) was added during this time.
6- The titration was completed within a total boiling time of 3 minutes by adding standard glucose, drop-wise, to decolourization of the indicator. (This is $V_{\text{Blank}}$)
7- The titrations were repeated until successive titrations agreed within 0.4mL.
8- Approximately 1.3 g of each honey sample was made up to 100 mL with water in a volumetric flask.
9- Pipette 10 mL of the sample into a conical flask and add 25 mL of the mixed Fehling’s solution.
10- The mixture was heated to boiling whilst stirring and maintained at boiling for 2 minutes adding methylene-blue indicator solution.
11- The titration was completed within the total boiling time of 3 minutes by drop-wise addition of standard glucose solution from the burette. (This is $V_{\text{Sample of RS}}$)
3) **Total Sugars:**
1) A measured amount (10 ml) of the dissolved honey sample taken in a 100 ml beaker to which 0.5 g of citric acid was added and kept for hydrolyzation by boiling for 10 min (cover the beaker by watch glass during boiling).
2) The solution is then neutralized with saturated NaOH (10%) solution the end point detected by phenolphthalein (3 drops) to the pink color.

3) The solution then decolorized by 10% HCl.

4) This solution was then titrated against Fehling’s A and B as was done previously in case of reducing sugars. (This is $V_{sample \, TS}$).

**Calculations:**

1) **For reducing sugars:**

$$V_{diff} = V_{Bl} - V_{sample \, RS}$$

$$V_{diff} \times \frac{0.5 \times 50}{100 \times 10} = g \, RS \, in \, sample$$

$$% \, RS = \frac{g \, RS \, in \, sample}{Wt \, sample} \times 100\%$$

2) **For Total sugars:**

$$V_{diff} = V_{Bl} - V_{sample \, TS}$$

$$V_{diff} \times \frac{0.5 \times 50}{100 \times 10} = g \, TS \, in \, sample$$

$$% \, TS = \frac{g \, TS \, in \, sample}{Wt \, sample} \times 100\%$$

4) **For Sucrose:**

$$% \, Sucrose = % \, TS - % \, RS$$
**Experiment # 9**

**Determination of Copper in an Alloy**

*(Using Standard addition Method)*

The method described here has only moderate sensitivity, but it is relatively free from interferences. It is based on absorption of light at 800 - 900 nm by copper in strongly acidic solution. The absorption is strongest at 800 nm, but in the presence of nickel, the 870 nm wavelength is preferable. Nickel, chromium, iron, and cobalt in amounts up to 4% each, do not appreciably interfere. These percentages are above those normally contained in copper-based alloys. Tin will be largely precipitated as metastannic acid during the dissolution process. If this is removed by filtration, loss of copper with the precipitate must be guarded against when large amounts of tin are present.

The standard addition method is used for the analysis of the unknown. This increases the accuracy of the determination by minimizing matrix effects - it provides a means to avoid errors resulting from the presence of a substance that alters the response of the analyte.

- Preparation of several samples.
- These samples contain same volume of unknown analyte \( V_{\text{unk}} \) with \( C_{\text{unk}} \).
- Addition of different volumes of a standard solution \( V_{\text{std}} \) of known \( C_{\text{std}} \).
- Dilution to same final volume using blank of the standard solution.

*Note: each solution contains all of the matrix in the same concentration (same matrix effect), but in different concentration of standard.*
The principle is illustrated in Figure (1):

$A_0$ corresponds to the absorbance of the unknown, while $A_1...A_n$ correspond to absorbances of the same unknown with progressively larger amounts of added copper standard. Extrapolation of the line to zero absorbance gives the quantity of copper in the original sample. Note that the quantity, or concentration, of copper added is plotted on the x-axis.

![Figure (1)](image)

**Figure (1)**

**Apparatus:**

- 250-mL beaker
- burette
- 50-mL volumetric flask
- volumetric flask, 10 mL or larger
- spectrometer with cell

**Chemicals:**

- Copper
- nitric acid

**Procedure:**

*Preparation of standard solution.*

Prepare 100 mL of $5 \times 10^5$ ppm copper solution.
Sample treatment.

1) Accurately weigh (to 0.1 mg) 0.1 - 0.15 g of unknown into a 250-mL beaker or Erlenmeyer flask and add 10 mL of 1:1 HNO₃.

2) Boil the solution until it turns blue, cool, transfer quantitatively to a 100-mL volumetric flask and dilute to the mark.

3) Prepare a blank solution by following the same procedure, but omitting the brass sample.

4) Prepare 6 samples using standard addition as presented in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>V_{unk} (mL)</th>
<th>V_{std} (mL)</th>
<th>V_{T} (mL)</th>
<th>C_{Stnd} (ppm)</th>
<th>Abs. Reading</th>
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</thead>
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<td>50</td>
<td></td>
<td></td>
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<td>5</td>
<td>25</td>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measurements:

- Set the spectrometer to zero at 870 nm with the blank solution.
- Measure the absorbance of the six samples (Table 1).
- Prepare a plot as shown in the figure above.
- Determine the concentration of copper in the unknown and hence the percent copper in the alloy.
Note you can plot $V_{std}$ against absorbance and to determine the unknown concentration you can apply the following equation:

$$C_{unk} = \frac{b}{m} \times \frac{C_{std}}{V_{unk}}$$

*Where: $b$ is the y-intercept and $m$ is the slope*

--------------------------------------
Experiment #10

Quantitative Spectrophotometric Method for Determination of Total Iron in Wheat Flour

Principle:

The determination of total iron in foods usually includes the total combustion of organic materials leaving only the ash, which contains the mineral part of foods. This process transforms all iron present to the oxidized ferric form (Fe$^{3+}$). A solution of the ash is prepared using hydrochloric acid and the iron (III) is reduced to iron(II) using hydroxylamine hydrochloride. The ferrous ion (Fe$^{2+}$) can be determined spectrophotometrically by forming colored complexes using several chromogens that interact with iron (Fe$^{2+}$) an example is 1,10-phenanthroline.H$_2$O. The color reaction has to be performed under pH-controlled conditions suitable for the chromogen.

In order to reduce the competition by hydronium ions (H$^+$) for the ligand, a solution of 2 M sodium acetate is added.

Critical points:

1) Clean and wash all glassware following appropriate cleaning procedures for analysis of minerals.
2) All reagents have to be analytical grade with the minimum possible content of iron.
3) The water used has to be distilled and deionized, with less than 2µ Si/cm conductivity, or 10-6 (ohm. cm)$^{-1}$.
4) It is critical to maintain the pH of solutions between 5-6. If necessary, more sodium acetate can be added to increase the pH.
**Equipment and materials:**

Analytical balance  
Cuvets (1 mL capacity).

Furnace (Temperature > 500 °C)  
Funnels

Graduated cylinders  
Porcelain crucibles

Spectrophotometer UV/VIS  
Vortex mixer

Volumetric flasks (50, 100, 250 mL)

Volumetric and graduated pipettes

**Reagents:**

- Hydrochloric acid (HCl), 37%.
- Nitric acid (HNO₃) 65 %.
- Sodium acetate trihydrated, (CH₃COONa. 3H₂O).
- 1,10-phenanthroline-monohydrate, Mwt = 198.23.
- Hydroxylamine hydrochloride (NH₂OH.H Cl), Mwt = 69.49.
- Glacial acetic acid (CH₃COOH), Mwt = 60.05.
- Standards Solution for iron Ammoniacal Ferrous Sulfate, Fe \((\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}\), Mwt = 392.

**Solutions:**

1) **1,10-phenanthroline.H₂O:**

Dissolve 0.1 g 1,10-phenanthroline.H₂O in ca 80 mL H₂O at 80° C, let it cool down, and dilute to 100 mL. Store in a dark bottle under refrigeration. The solution is stable for several weeks. Discard if the solution turns lightly pink, indicating that it has been contaminated with iron.
2) **Acetate Buffer-2 M:**
In a 500 mL beaker add 68 g sodium acetate trihydrate, and dissolve in approximately 100 mL of deionized water. Add 60 mL of glacial acetic acid and dilute to 500 mL. Transfer the solution into a glass flask with hermetical cover. The solution is stable for indefinite time.

3) **Hydroxylamine Hydrochloride –10 %:**
Add 10 g of hydroxylamine hydrochloride into a beaker, and dissolve with 100 mL of deionized water with the aid of a glass rod. Transfer the solution into a glass flask with hermetical cover. The solution is stable for indefinite time.

**Standard solutions:**

1) **Primary Standard Solution of Iron – 1000 mg/L.**
Dissolve 3.512 g of Fe(NH₄)₂(SO₄)₂·6H₂O in distilled water, and add a few drops of concentrated HCl. Dilute to 500 mL in a volumetric flask. Transfer the solution to a plastic bottle. This solution is stable for indefinite time, unless a light pink color is observed indicating contamination.

2) **Secondary Standard Solution of Iron-100 mg/L.**
Into a 100 mL volumetric flask pipette 10 mL of the Primary Standard Solution (1000 mg/L). Add 2 mL concentrated HCl. Fill with distilled water up to the 100 mL mark. Transfer the solution to a plastic bottle and store it in a cool dry place. This solution is stable for about 6 months.

**Procedure:**

**A) Dry digestion (ashing):**

1) Clean the porcelain crucibles, and label using a high-temperature proof marker.
2) Dry crucibles in the oven at 110 °C and cool in a dessicator. Repeat until constant weight is attained.
3) Take about 100 g of the flour and grind in a mortar and pestle and mix well.
4) Weigh 1 g of the previously homogenized sample in duplicate. Weigh by difference directly into the crucibles using an analytical balance and record the weights accurately to 3 decimals (0.001 g).
5) Place the crucibles into the muffle furnace at 550 °C and heat for 6 hours.
6) Turn the oven off and wait until the temperature has decreased.
7) The ashing is complete when a white or grayish ash is obtained. If this is not the case, continue the ashing until white/grayish ash is obtained.
8) Let the crucibles cool down for 5 minutes and place in a dessicator for 1 hour until they reach room temperature.

b) Preparation of the ash solution:

1) Add 5 mL of concentrated HNO₃ to the crucible, pouring the acid onto the inside walls of the crucible.
2) Evaporate the acid by heating the crucibles on top of a hot plate at low temperature, solution should not boil.
3) Dissolve the remaining residue by adding 2 mL of concentrated HCl, and heat for few minutes, taking care that the solution does not spill out the crucible.
4) Let the crucible cool down and transfer the solution quantitatively into a 50.0 mL volumetric flask. Wash crucible with distilled water and bring to volume with deionized water.

c) Standard Solutions for the Calibration Curve:
1) Solutions (50 mL) for the calibration curve will have iron levels from 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 15.0 mg/L (ppm) (Table 1).

2) Into 50 mL volumetric flasks, pipet the amounts of the Secondary Standards Solution (10 mg/L) – you should calculate the required amount.

3) Add 1.0 mL of hydroxylamine hydrochloride solution, mix well and let it stand for 5 minutes.

4) Add 5.0 mL acetate buffer and 4.0 mL of 1,10-phenanthroline to each flask. Mix well and color will start developing.

5) Let stand it for 30 min and then make up to volume (25 mL) using deionized water.

d) Determination of iron.

1) Pipet 15.0 mL of the unknown sample solution into 50.0 mL volumetric flask, then add 1.0 mL of hydroxylamine hydrochloride solution, mix well and let it stand for 5 minutes.

2) Follow the same procedure as for the samples.

e) Measurement:

1) Turn on the spectrophotometer 15-20 minutes before using it to warm up.

2) Adjust the wavelength to 510 nm. Set the mode to Absorbance.

3) Set the instrument to zero Absorbance using deionized water.

4) Read the absorbance of the 0.0-mg/L standard solution (blank) and record the absorbance.

5) Read the absorbance for the standard solutions and flour sample solutions.

6) If color intensity of the samples is too high, make appropriate dilution of the sample solutions and record the absorbance again.
### Table 1

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<th>Sample</th>
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<th>V (100 ppm) needed</th>
<th>Total volume</th>
<th>Absorbance</th>
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<tr>
<td>Unk 2</td>
<td>?</td>
<td>10 mL Unk</td>
<td>50</td>
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### Calculation:

1) Plot a graph of the absorbance values of the standard solutions (y-axis) against concentration (x-axis) and obtain the equation of the standard curve. The equation will be similar to the one obtained for soluble iron.

2) Calculate the concentration of soluble iron in the sample solution solving the standard curve equation for x.

3) Calculate the concentration of soluble iron in the flour sample (mg/kg).

4) Compare your results with the standard recommended values.
Zinc makes up about 75 ppm (0.0075%) of the Earth’s crust, making it the 24th most abundant element. Zinc is necessary for the functioning of more than 300 different enzymes and plays a vital role in a large number of biological processes. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division. Zinc also supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system.

Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement. Zinc is also found in many cold lozenges and some over the counter drugs sold as cold remedies.

The body doesn’t readily store zinc, so you need to get some every day—but only a small amount. The recommended daily allowance (RDA) is 8 mg per day for women. That number rises to 11 mg for pregnant women, and 12 mg for nursing mothers. Meanwhile, vegetarians may need to take in as much as 50% more than the RDA—the body absorbs less zinc from plant-based foods than from meat sources (a term called bioavailability).

**Objective:**

In this experiment, you will find the concentration of zinc in a zinc supplement tablet by EDTA titration, with the assumption that EDTA reacts 1:1 with metal (Zn$^{2+}$) ions.
**Equipment**

150-mL Erlenmeyer flask (3)  
50-mL Burette  
Ring-stand and hardware  
400-mL Beaker  
10.0-mL Vol Pipette  
100-mL grad cylinder  
250-mL Volumetric Flask  
Hot plate

**Chemicals**

Eriochrome Black T:  
Buffer (pH 10.0):  
Unknown: Zinc supplement tablet

Submit a clean 250-mL volumetric flask to the instructor so that an unknown zinc solution may be issued.

**Theory:**

This experiment is an example of a classic titrimetric analysis. This titration is known as a complexometric or chelometric titration because the titrant (ligand) reacts with the analyte (metal ion) to form a complex (chelate). A chelate is a ligand that has two or more sites that bind to the central ion. EDTA \([\text{ethylenediaminetetraacetic acid,} \ C_{10}H_{16}N_{2}O_{8}, (\text{HOOCCH}_2)_2N-\text{CH}_2\text{CH}_2-N(\text{CH}_2\text{COOH})_2, \ MW = 292.24 \ g/mol, \ often symbolized by H}_4Y]\) is an excellent chelating agent. It forms very strong 1:1 complexes with almost every divalent and trivalent metal ion depending on solution conditions.

![structure of EDTA](image)

The reaction is as follow, ignoring charges for the moment,

\[
\text{EDTA} + \text{M} \leftrightarrow \text{M DTA}
\]
Although it is equilibrium, the reaction lies very far to the right. The equilibrium formational constants, $K_f$, are on the order of $10^8 - 10^{25}$ depending on the metal and other conditions.

EDTA itself is a tetraprotic4-acid; it has 4 ionizable protons (usually represented as $H_4Y$) with p$Ka$'s = 1.99, 2.67, 6.16, 10.26. In its fully ionized form, $Y^{-4}$, the four acetate groups and the lone pairs on the two nitrogen makes it a hexidentate ligand that wraps itself very tightly around a metal ion. Usually, titrations are performed in basic solution, roughly pH 8-11.

The fully protonated form, $H_4Y$, is only sparingly soluble in water, so the standard form of EDTA used analytically is usually the disodium salt $Na_2H_2Y\cdot2H_2O$ (372.24 g/mol), which is much more soluble and available in primary standard purity, except for a small (about 0.3%) amount of adsorbed water.

Concentrations of metals such as zinc, calcium and magnesium can be determined by titration with a standard solution of ethylenediamminetetraacetic acid, EDTA. The EDTA is a complexing, or chelating agent used to capture the metal ions. For example, when calcium in water is bound to EDTA, the water is now softened. The term “soft” water refers to treated water that cannot precipitate the mineral residues (limescale or soap scum) found in "hard" water, because the ions now bind EDTA, the chelating agent.

**In this lab:**

You will be asked to determine the amount of zinc in a zinc supplement tablet and an unknown solution. EDTA grabs all the metal ions in the water, not just the $Zn^{2+}$ ions. This may result in a value that is not truly the concentration of $Zn^{2+}$ ions and causes an experimental error of about 1%.
Procedure.

Skip this step if the EDTA titrant has been prepared for you.

Preparation of EDTA, 0.01 M:

This solution must be prepared at least one day ahead of time, a week is preferable, to ensure that the solute is completely dissolved. EDTA solutions are prepared at an approximate molarity, and then standardized against a solution of a primary standard such as CaCO$_3$.

5) Dissolve about 3.8 g of the dihydrate of the disodium salt (Na$_2$H$_2$Y.2H$_2$O) and 0.1 g MgCl$_2$ in approximately 1 L of deionized water in a large beaker or a 1-L plastic bottle using a magnetic stirrer. A small amount of sodium hydroxide can be added if there is any difficulty in dissolving the EDTA. Try not to exceed 3.8 g of the disodium salt because much more than this dissolves only with difficulty.

6) Before use, the EDTA solution should be filtered using a Buchner funnel and suction filtration.

7) Store the solution in a clean, labeled 1-L plastic bottle that has been rinsed with deionized water. Never store reagent solutions in volumetric flasks.

Ammonia/Ammonium Chloride Buffer Stock Solution, pH 10:

Each titration will require the addition of pH 10 ammonia buffer. The stock buffer solution has been prepared for you, and you should not have to prepare it. The appropriate quantity (7-8 mL) is dispensed directly into your titration flask from the plastic Repipet® repetitive dispenser. The buffer should only be added immediately before you titrate an individual sample.

1) Dissolve 64.0 g of ammonium chloride in 600 mL of concentrated ammonia (14.8 M, 28% NH$_3$).

2) Slowly and carefully add 400 mL deionized water with stirring. This should be sufficient for over 120 titrations.
**Calcium Standard Solution.**

A CaCO₃ solution is prepared as a primary standard for Ca and used to standardize the 0.01 M EDTA titrant.

1) Tap out approximately 1 g of predried analytical-reagent-grade CaCO₃ in a weighing boat. Accurately weigh (to within ± 0.1 mg) approximately a 0.25-g sample by difference into a 150- or 250-mL beaker.

**NOTE: NEVER transfer chemicals inside an analytical balance.**

2) Add about 25 mL deionized water and then slowly add concentrated HCl dropwise with periodic stirring until the sample dissolves completely. Then add 2 drops more. Keep the beaker covered during the entire dissolution process. Mild heating will speed the dissolution. **Do NOT boil; this will spatter the calcium solution and lead to losses.**

3) Transfer the solution quantitatively into a 250-mL volumetric flask. Rinse the beaker thoroughly with deionized water, and carefully dilute to the mark with an eye dropper or with careful use of your wash bottle. Mix thoroughly.

Because this Ca²⁺ standard solution is used to standardize the EDTA titrant, it must be prepared very carefully so that you know its exact molarity. Therefore, an exactly known (to ± 0.1 mg) mass of CaCO₃ must be weighed out, dissolved completely, and transferred quantitatively into the 250-mL volumetric flask. This is critical.

**Standardization of the EDTA Solution:**

1) Use clean dry 50-mL burette rinsed with 2 mL of standard EDTA solution then fill the burette with your standard EDTA solution. Check to see if any air bubbles are trapped in the tip of the burette.

2) Pipet 25.00-mL aliquots of your standard Ca²⁺ solution into each of three or four 250-mL Erlenmeyer flasks.
3) Add 7-8 mL of pH 10 buffer from the Repipet® dispenser, 15 mL of deionized water, and 3 drops of Eriochrome Black T indicator, immediately prior to titrating a sample. The solution should be a pale pink. Do not add more indicator to make the solution darker as this can cause problems with the endpoint. Titrate the solution immediately with EDTA against a white background until the LIGHT PINK solution turns a LIGHT SKY BLUE. Read the final volume at least twice.

*Titrations must be performed swiftly (but carefully) because the NH₃ will evaporate to some degree and thus the pH of the solution will change.*

In general, the faster the titrations are performed the better the results will be, as long as the endpoint is not overshot due to excessive haste. The endpoint color change is rather subtle, and sometimes it is slow, so you need to be careful at the end. If you are having trouble with the endpoint color change, see Note 1 at the end of the report for the preparation of "before" and "after" flasks.

- Calculate the molarity of the EDTA solution from the volume of EDTA used in the titration of each aliquot.
- The values (MEDTA and titration volumes) should all agree very closely, to within about +0.4% relative standard deviation. If not, titrate additional aliquots until better agreement is reached.
- Outlying values can always be rejected for cause.

**Titrating the Zinc tablets:**

The instructions are based on a target of 1 tablet (100mg) of zinc supplement tablet. The tablet should be crushed to powder to mask their brand identity.

1) You will be given an unknown for a specific brand of zinc supplement.

   Weigh out about 0.1 grams to the nearest 0.1mg. Place the powder into a clean 250mL Erlenmeyer flask and then add about **30 mL** of
deionized water, using the flask volume markings. Heat the contents gently on a warm (not boiling) hot plate if necessary for a few minutes until the tablet disintegrates. Depending on the tablet, some binders fillers might not dissolved. This is okay as the zinc will be in solution.

2) Add 7-8 mL of the pH 10 buffer solution and add 3 drops of Eriochrome Black T immediately prior to titrating a sample.

3) Refill your burette with EDTA solution as necessary. Titrate as previously mentioned above, to the end-point color change. Repeat until you are confident that you have satisfactory titration results for three (3) samples.

4) Upon completion of the experiment, discard all solution in a chemical waste bottle and wash out the glassware. Be sure to dry your burette in

IMPORTANT NOTE

1) Eriochrome Black T Indicator. The color change of Eriochrome black T at the endpoint is rather subtle. It is not an abrupt change from deep red to a dark blue; but rather it is from a light red (or pink) to a pale blue. At least one trial titration is recommended. (You can always discard a "bad" value when you know there is a definite reason for its being bad. Make sure you indicate a possible problem in your notebook at the time you observe it.)

2) Sometimes the Eriochrome black T solution goes bad because of air oxidation. If the endpoints seem very indistinct or slow to you, try a fresh bottle of indicator. Alternatively, try adding a small amount of solid Eriochrome black T mixture (1 g indicator ground with 100 g NaCl). A small amount on the end of a spatula is sufficient.

Calculations:

Mass of zinc in a vitamin supplement.
Approximately 0.10 g of a supplemental tablet was used in this analysis.
1) Calculate the moles of EDTA to reach the equivalent point for the titration of zinc tablet based on the volume used.

The reaction of Ca\(^{2+}\) ions with Na\(_2\)H\(_2\)EDTA takes place with a 1:1 stoichiometric ratio:

\[
\text{Zn}^{2+} + \text{Na}_2\text{H}_2\text{EDTA} \leftrightarrow \text{ZnH}_2\text{EDTA}.
\]

*At the end point of the titration:*

\[
1 \text{ mol of } \text{Zn}^{2+} = 1\text{mol of } \text{Na}_2\text{H}_2\text{EDTA}.
\]

2) Convert of moles of EDTA to moles of zinc.

3) Calculate the mass of zinc per 0.100 g of the supplemental table.

4) Calculate the % w/w of zinc in 0.100 g sample.

**Statistical Analysis:**

1. Report the mean, medium, standard deviations (s), relative standard deviation (RSD), for your results.
Experiment # 12

Simultaneous Determination of Benzoic Acid and Caffeine Concentration in Soft Drinks by HPLC

Introduction:

Soft drinks frequently contain a number of additives that affect the beverage’s taste and characteristics. Caffeine, one popular ingredient, is a natural xanthine alkaloid stimulant that exists in many plants as a natural insecticide, including kola nuts, coffee beans, cacao beans, and tea leaves. In some cases, it’s extracted from the natural ingredients as part of the beverage-making process (e.g. in coffee and tea and in cola beverages). In other cases, it’s added by the manufacturer (e.g. in some citrus-flavored sodas). Often, even if Caffeine is naturally present, it is elevated or controlled at a consistent level by the manufacturer (e.g. in many colas). Benzoic Acid is also added to many soft drinks and other foods as a preservative against microbial growth. Diet soft drinks often contain low calorie artificial (man-made) sweeteners in place of sugar or corn syrup. We will analyze for Caffeine, Benzoic Acid in a soft drink. Table (1) gives information of five additive compounds.
HPLC (Figure 1) is one of the most popular and widely used chromatographic techniques today. This method uses a solid or “bonded” stationary phase and a liquid mobile phase to separate mixture components in time. The method yields information about the identity of mixture components (qualitative information) and their concentrations (quantitative information).
In this method, stationary phase particles (usually SiO$_2$) are coated with a chemically-bonded layer of some type of non-polar molecule. Commonly used bonded layers include 18 and 8 carbon-long straight chain alkanes (C18 and C8) and phenyl groups. The mobile phase is typically water mixed with some fraction of miscible, polar organic solvent (usually Methanol, Acetonitrile, or THF). During the separation, analyte molecules partition between the mobile phase and the bonded layer of stationary phase. Since relatively non-polar molecules will dissolve more easily into the stationary phase, they will elute last. Relatively polar compounds will not interact as strongly with the stationary phase and will therefore elute first. This mode works well for the separation of Water-soluble, non-volatile organic compounds. Since these compounds are not usually amenable to analysis by gas chromatography (GC), reversed phase HPLC is an excellent compliment to that method.
Materials and Methods.

Preparation of standard solutions.

1) Prepare a mobile phase with a ratio of 60/40 methanol/water.
2) All solutions should be prepared using HPLC grade solvents.
3) Sodium phosphate buffer: prepare 0.05M from sodium phosphate and the solution adjusted to a pH of 5.0.
4) Combine 600 mL of methanol with 400 mL of phosphate buffer. Degas in ultrasonic bath for 2 min and filter through 0.4 µ polyvinylidene fluoride filter.

Benzoic acid standard solutions:

1) Prepare 100 mL stock standard solution of benzoic acid of 1000 ppm: dissolve accurately benzoic acid (0.1 g) in the mobile phase up to a volume (100 mL) and degassed by sonication, then stored and chilled at 1.8°C.
2) Prepare standard solutions of benzoic acid of 50, 100, 150 and 250 ppm (in 50 mL) by dilution from the original sample stock solution.

Caffeine standard solutions:

3) Prepare 100 mL stock standard solution of caffeine of 1000 ppm: dissolve accurately caffeine (0.1 g) in the mobile phase up to a volume (100 mL) and degassed by sonication, then stored and chilled at 1.8°C.
4) Prepare standard solutions of benzoic acid of 50, 100, 150 and 250 ppm (in 50 mL) by dilution from the original sample stock solution.

Preparation of soft drink sample:

1) Take 10 mL of each soft drink sample containing benzoic acid and caffeine ingredients and dilute with the methanol/water mobile phase to 50 mL.
2) Degas the diluted sample by vacuum to remove effervescence and filter (through pore size 45µm) to remove impurities.
**HPLCL Instrument conditions:**

1) Column: C-18 RP  
2) The injector: 20 microliter loop  
3) The ultraviolet light detector: set to a wavelength of 270 nm.  
4) Initial conditions set at approximately 4,000 psi and 1.5 mL min⁻¹ with 100% of solvent running for pre conditioning.  
5) The column equilibrated with a non-buffered 60/40 methanol/water solution before and after each running session.  
6) Flow rate: 1mL/min  
7) Elution time: 15 min.  
8) Column temperature: ambient  

**Procedure:**

1) Setup the HPLC instrument.  
2) After the instrument has been running for about 15 min, inject standard solutions of benzoic acid and caffeine.  
3) From the chromatograms determine the retention times for benzoic acid and caffeine and determine the peak area for each standard.  
4) Inject the prepared soft drink sample in the same conditions.  
5) Draw calibration curves for both benzoic acid and caffeine by plotting the peak area versus the standard concentrations.  
6) The relationship between concentration and peak area should be linear and determine the regression factor of each curve.  
7) Identify caffeine and benzoic acids in the samples by comparing the known retention times of the standard pure solutions to the retention times that were displayed on the chromatogram of the sample.  
8) From the calibration curves and according to the peak area and dilution factor, determine benzoic acid and caffeine contents in the beverage sample in ppm and mg/100 mL.
Results:

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<tr>
<th>Benzoic acid</th>
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<td>C std</td>
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Experiment #13

Spectrofluorimetric Determination of Paracetamol in Tablet

Introduction:
Paracetamol (N-acetyl-4-aminophenol) is widely used as an active ingredient in pharmaceutical preparations. This substance is mainly used as an alternative to aspirin because of its analgesic and antipyretic activity.
In this experiment a series of standard solutions of paracetamol are prepared; the fluorescence of the standards and the samples are measured; and the working curve method is used to determine the concentrations of paracetamol in the sample solutions. The concentration is used to calculate the mg and percentage of paracetamol tablet.

Apparatus:
100 mL beaker  
mortar and pestle  
Filter paper (medium porosity)  
Glass funnel  
Three 100 mL volumetric flasks  
Six -50 mL volumetric flask  
Wash bottle

Chemicals:
Paracetamol tablets  
Paracetamol standard (reagent grade)  
NH₄OH/ NH₄Cl buffer solution pH 10.5

Procedure:
2- Prepare 1000 ppm stock solution of paracetamol in 100 mL volumetric flask using deionized water.
3- This solution should be protected from light for better conservation.
4- Use ultrasonic agitation to help dissolving the analyte.
5- For calibration curve prepare six standard solution of 10, 12, 14, 16, 18 and 20 ppm in 50 mL volumetric flasks by dilution from the
previous stock standard solution. Before dilution to the mark add 1 mL of buffer; NH$_4$OH/ NH$_4$Cl solution to all samples for keeping the pH equal to 10.5. Prepare a blank solution with buffer in deionized water.

6- Obtain a paracetamol tablet from the instructor. Record sample number on the tablet or the brand and/or manufacturer’s name if it is available.

7- Place the tablet in a clean, dry mortar. Use a clean pestle to grind the tablet into a powder. Weigh about 0.1 g of the powder to the nearest 0.1 mg into 100 mL beaker dissolve in deionized water and then filter in 100 mL volumetric flask and complete to the mark.

8- Take 1 ml of the paracetamol sample solution and 1 mL buffer; NH$_4$OH/ NH$_4$Cl and then dilute to 100 mL volumetric flask.

3- Based on spectra of paracetamol, set the excitation wavelength at 246 nm and the emission wavelength at 366 nm.

4- The instructor will provide the operating instructions for the fluorometer.

5- Measure and record the relative fluorescence of each of the standard and sample solutions.

Calculations:

1- Prepare a working curve.

2- From the working curve, and the dilution factor determine the concentration of paracetamol sample solution.

3- Use the paracetamol concentration and the mass of the tablet which was used to prepare the solution to calculate the amount in mg and the percentage of paracetamol in the tablet.