Abstract
An important aspect of organic chemistry is the isolation and purification of compounds from natural sources. This requires sound chemical knowledge and considerable experimental skill. However, the separation of caffeine from tea leaves is especially easy, since it can be done with simple techniques and since the concentration of caffeine is relatively high. This experiment introduces a number of experimental techniques commonly used in organic chemistry such as extraction and sublimation.

Caffeine, $\text{C}_8\text{H}_{10}\text{O}_2\text{N}_4$ is a compound of the large class called alkaloids which are usually defined as nitrogenous plant products having a marked physiological action when administered to animals. Many alkaloids are extremely difficult to synthesize; hence plants are still important commercial sources for them. Caffeine present in tea leaves and in coffee to the extent of about 4%. Tea contains two alkaloids, theobromine and theophylline, which are similar to caffeine in chemical structure. Caffeine stimulates the heart and respiratory system, but theophylline and theobromine relax the smooth muscles. Thus, tea relaxes some physiological systems while stimulating others. By way of contrast, coffee contains no relaxing alkaloids and it has a more stimulating effect.

As a matter of general interest, the daily consumption of less than 200 mg of caffeine is considered to be minor while a daily consumption of more than 700 mg will almost guarantee an ulcer. The following levels of caffeine have been specified by the British Columbia Alcohol and Drug Commission.

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Caffeine Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average cup of regular coffee</td>
<td>90 – 150 mg</td>
</tr>
<tr>
<td>Average cup of instant coffee</td>
<td>60 – 80 mg</td>
</tr>
<tr>
<td>Average cup of tea</td>
<td>30 – 70 mg</td>
</tr>
<tr>
<td>Bottle of cola</td>
<td>30 – 45 mg</td>
</tr>
<tr>
<td>Average chocolate bar</td>
<td>30 mg</td>
</tr>
<tr>
<td>Stay-awake pill</td>
<td>100 mg</td>
</tr>
</tbody>
</table>
As with other natural material, tea leaves contain a large number of organic substances, many of which have complex structures. Some of the major groups of organic substances present are:

Nitrogen compounds: alkaloids, amino acids, proteins
Polyphenols and their derivatives
Pigments: chlorophylls, anthocyanins, flavones
Carbohydrates: cellulose, sugars, starch
Lipids
Enzymes
Vitamins

In the isolation of caffeine, the problem is to find a method suitable to pick out this one compound in an efficient way, without undue losses, out of a host of other compounds, some of which may be chemically very similar to caffeine. This is done by selective extractions and precipitation and removal of unwanted substances. These techniques take advantage of the fact that caffeine is soluble in hot water and in methylene chloride at room temperature but, unlike some other materials found in tea leaves, it forms no insoluble lead salts with a lead acetate solution. Caffeine is itself basic and does not react with 5% sodium hydroxide as do the organic acids present in tea leaves. The unwanted acids are extracted into an aqueous layer in the form of sodium salts.

A simplified summary of the entire procedure is given on page 4 in the form of a flow sheet.

**Purpose**
Crude caffeine will be isolated from aqueous tea solution by means of solvent extraction with Methylene chloride.

**Materials**

| 600 mL beaker | 10% Lead acetate |
| 50 mL beaker | 5% Sodium hydroxide |
| Electric hotplate | Methylene chloride |
| Tongs | Celite |
| Filtration apparatus | 2 tea bags |
| Separatory funnel | Boiling chips |
| Eyedropper | |
| Melting point apparatus | |
| 25 mL graduated cylinder | |
| 5 x 100 mL beaker | |
| Filter paper | |
| Scale | |
| 100 mL graduated cylinder | |
**Procedure**

1. Bring about 150 mL of tap water to a gentle boil (use a boiling chip) on an electric hotplate in a 600 mL beaker.

2. Obtain a clean, dry 50 mL beaker and record the beaker number and its mass on the observation sheet provided.

3. At the onset of boiling, add two tea bags and sustain the boil for ten minutes.

4. Reduce the heat and remove the tea bags with tongs. Using a 100 mL graduated cylinder, add 40 mL of 10% Lead acetate and boil the mixture for 15 minutes.

5. Using the top loading scale, prepare a slurry of about 5 g Celite and about 20-30 mL of water in a 100 mL beaker. Dampen a filter paper on a Buchner funnel and turn on the vacuum. Swirl the slurry until all the particles are suspended and then quickly pour the slurry into the filter paper. It should form an even dense cake on the filter paper. Discard the water in the filter flask.

6. Vacuum filter the hot tea mixture until the filtrate is clear and free of particulate matter. Be careful not to pour the hot tea too quickly in the same area since this will spread the slurry and render it ineffective. Pour the filtrate from the vacuum flask into a clean 100 mL beaker.

7. Evaporate the filtrate by boiling it in an opened 100 mL beaker until the volume is reduced to about 50 mL. If the boiled down solution is turbid, filter it until it is clear. Cool the solution to room temperature before continuing the procedure.

8. Discard the spent tea bags and filter paper in the trash bins.

9. Add 20 mL of Methylene chloride using a 25 mL graduated cylinder to the cooled tea extract in the separatory funnel (be sure that the stopcock is closed). In the solvent extraction caffeine and related impurities are concentrated in the Methylene chloride (the bottom phase).

10. Agitate the mixture gently several times. **DO NOT** shake too vigorously during the extraction as an emulsion may be formed, from which it is tedious and often very difficult to recover the caffeine.

11. Withdraw the Methylene chloride layer into a clean 100 mL beaker. Extract the aqueous phase a second time by adding 15 mL of Methylene chloride to the separator funnel. Extract the second Methylene chloride and add it to the first batch in the 100 mL beaker.

12. Discard the aqueous phase into a different 100 mL beaker and save it in case of a mistake.

13. Take the Methylene chloride from the 100 mL beaker and extract it with 20 mL of 5% Sodium hydroxide using the separator funnel. Caffeine remains in the Methylene chloride phase (the bottom phase). Collect this in a clean, dry 100 mL beaker. Discard the aqueous phase into a different 100 mL beaker and save it in case of a mistake.

14. Take the Methylene chloride from the 100 mL beaker and extract it with 15 mL of cold water using the separator funnel. Collect the Methylene chloride (the bottom phase) into a clean, dry 100 mL beaker.
Tea Leaves

Boiling water extraction

Extract

Lead acetate precipitation, filtration

Residue (Fibrous plant matter)*

Filtrate

Precipitated polyphenols*

Concentration, filtration

Filtrate

Substances with low water solubility*

Methylene chloride extractions

Methylene chloride layer

Aqueous layer (water soluble substances)*

NaOH solution extraction, water wash

Methylene chloride layer

Aqueous layer (base soluble substances)*

Drying filtration, evaporation

Residue (crude caffeine)

Distillate (lost to atmosphere)

Sublimation

Sublimate (pure caffeine)

Residue (theophylline etc.)*

* indicates materials to be discarded
15. Filter the Methylene chloride using a conical funnel. The filter paper in this filtration, and in this filtration only, must be wetted with Methylene chloride using an eyedropper, whereupon Methylene chloride will pass through the filter paper and any water will remain as droplets on the paper. Collect the Methylene chloride into the numbered 50 mL beaker that you weighed at the beginning of the lab.

16. In the fume hood with the door closed, heat the filtered Methylene chloride solution in the 50 mL beaker until all the Methylene chloride evaporates. The residue is crude caffeine. Keep an eye on the beaker all the time because once all the Methylene chloride evaporates off, the crude caffeine residue will sublime off very quickly. Therefore, remove the 50 mL beaker right away after all the Methylene chloride is gone. Let the product cool down.

17. Take your 50 mL beaker with the crude caffeine to the scale and record its mass on the observation sheet provided.

18. Using a melting point apparatus, find the melting point of your sample and record your findings on the data sheet provided.

19. Clean and store all glassware used. Hand in your sample and observation sheet to the lab supervisor.
Observations

Names: _______________________________________

_____________________________________

Beaker Number: _____

Table 1 – Mass of Caffeine Extracted

<table>
<thead>
<tr>
<th>Object Weighed</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of 50 mL beaker</td>
<td></td>
</tr>
<tr>
<td>Mass of 50 mL beaker and caffeine</td>
<td></td>
</tr>
<tr>
<td>Mass of caffeine</td>
<td></td>
</tr>
</tbody>
</table>

Melting Point of Product: __________ °C

Conclusion

The evaluation for this experiment will be based entirely on the mass extracted and the melting point of your product.
Appendix
I: Preparative Filtration

In preparative procedures filtration can be carried out with filter paper and a conical funnel. After a filter paper of proper size and porosity has been chosen, it is folded in half and creased. A second fold exposes a ten degree segment as shown below. Open the larger segment and place it in the conical funnel. Hold it in place until the paper is wetted with a few drops of the solution to be filtered. Wet filter paper is very fragile and easily ripped. Do not fill beyond the lip of the filter paper and always pour from a beaker along a stirring rod with the tip of the stirring rod close to but not touching the filter paper.

Vacuum filtration is carried out with an apparatus as is illustrated below.
The water pump provides a partial vacuum in the apparatus. External air pressure forces the liquid portion through the filter. Because force is used the filter paper must be carefully seated in place before the filtration begins. This is done as follows. Turn on the water tap to begin pumping. Open the air bleed and put the dry filter paper in place. Be certain that all the holes in the Buchner funnel are covered. Wet the filter paper and close the air bleed. Begin filtration as soon as air pressure causes the filter paper to become firmly sealed onto the bottom of the Buchner funnel. Wrinkled filter paper cannot form a proper seal. Pour into the middle of the filter; otherwise the edge of the filter paper may be lifted.

It may be necessary to apply firm hand pressure to the Buchner funnel so that the filtervac (a flat rubber ring) will seal onto the filter flask. If vacuum is not attained within a few seconds, look for possible air leaks elsewhere. The trap prevents water from being drawn into the primary filter flask in case the water pressure falls. Always break the vacuum by opening the air bleed.

II: Quantitative Filtration

For quantitative filtration the Buchner funnel, filter paper, and filtervac are replaced by a sintered glass filter crucible and adaptor. (Filter paper has a highly variable moisture content and cannot be used for quantitative work unless it can be burned away at high temperatures without decomposing the precipitate).

The sintered glass filter crucible is rinsed with deionized water, put in a 100 mL beaker, and oven dried at an elevated temperature for one hour. (Label by writing on aluminum foil). After removing from the oven, the beaker is tightly covered with aluminum foil to avoid needless exposure to atmospheric water and dust. When cooled to room temperature (about twenty minutes), it is carefully weighed on the analytical balance. It is never touched with bare fingers, set on dirty surfaces, or needlessly exposed to atmospheric dust. After it has been dried it must be handled with a paper strip as is shown in the illustration below.

Before seating the filter crucible in its holder, be sure the holder is free of dust, especially loose rubber particles.
In quantitative work it is absolutely essential that no discernible grain of precipitate is lost. To this end, never fill the filter crucible more than half full. The precipitate will creep wherever the filter crucible is wet; therefore the upper portion must remain dry.

Care is required to transfer all of the precipitate from the original container to the filter crucible. Consequently the container must be washed out several times with a suitable washing solvent, and with each wash, the rubber policeman is used to scrape down the walls of the container.

While filtering never allow the precipitate to dry. Keep the precipitate in the filter crucible covered with a minimum volume of the washing solvent. Open the air bleed to gain working time. Let the precipitate suck dry after all other procedures are complete.

In quantitative work the filter crucible and collected precipitate are oven dried, cooled and weighed on the analytical balance in the manner previously described.

III: Solvent Extraction

The separatory funnel is used for all simple solvent extraction processes. When two immiscible liquid phases are shaken together, components originally dissolved in one of the phases are redistributed over the two phases. It is often possible to select the solvents or phases so that one phase contains the wanted compound and the other phase contains the unwanted compounds.
The solvent mixture is shaken by holding the separatory funnel as shown above, by securing the stopper with the index finger of one hand and the stopcock (handle pointing upward) with the other hand. The funnel stem is pointed obliquely upward. At first, the pressure is released by carefully opening the stopcock while the funnel is held in an inverted position. Shaking and venting has to be repeated until the pressure in the funnel remains unchanged. A total shaking time of about one or two minutes is sufficient for each extraction. Do not shake while the stopcock is opened. Vent with care so that solvent droplets are not blown onto fellow workers.

Allow the mixture to separate with the separatory funnel supported in a ring clamp. If an emulsion forms stir the contents of the separatory funnel with a glass rod until there are two separate layers. Remove the stopper before attempting to withdraw the heavy phase from the separatory funnel. Violent shaking may lead to an unbreakable emulsion or worse yet a broken separatory funnel. It is wisest and safest to begin slowly.

Always return the separatory funnel to the ring stand when not in use; never lay it on the bench top. Store the separatory funnel with a small piece of paper between the ground glass stopper and the neck of the funnel. Otherwise the stopper may become jammed in place.