EXTRACTION - ISOLATION OF CAFFEINE FROM TEA[1]

Introduction

During the first week of lab, we learned about the process of recrystallization – a key purification technique used by organic chemists. Prior to purification there is often a "work-up" step that employs specific separation techniques. Extraction is one such technique whereby one or more molecules from a mixture are selectively dissolved in an appropriate solvent and separated based on their relative solubilities. Extraction can include removal of soluble compounds from a solid, but most often involves the transfer of compounds from one liquid to another. The technique is based on the idea that "like dissolves like."

Reading Assignments & Videos

Techniques:

- **Extraction & Drying agents**: Mohrig[2], p. 49-61, 71-72
- **Labflow videos posted under Lab 3 section**

Chemistry:

- Acids & Bases: Loudon[4], Chp 3.4 – 3.7
- Solvents: Loudon[4], Chp 8.6

Polar molecules are likely to be soluble in water, a polar solvent. Non-polar molecules will be far less soluble in water and instead will dissolve in non-polar organic solvents like dichloromethane (CH_2Cl_2) or diethyl ether (Et_2O). When a non-polar organic solvent, such as ether, and water are combined two layers are formed – similar to oil and water. The solvents are said to be *immiscible*. If a mixture of compounds (such as sodium dodecanoate and naphthalene, Fig. 1) is introduced to the mixture of solvents the compounds will migrate to the layer in which they are most soluble. The sodium dodecanoate is more polar than naphthalene (what makes it polar?) and therefore most of it will move to the water layer. Alternatively, naphthalene is more non-polar and most of it will distribute itself into the ether layer. The ether and the water layers can be physically separated (with the help of a separatory funnel) and in the process most of the dodecanoate salt is separated from the naphthalene. The efficiency of the separation is affected by the solubility of each compound in the solvent, the amount of solvent used, and the number of times the extraction is performed.

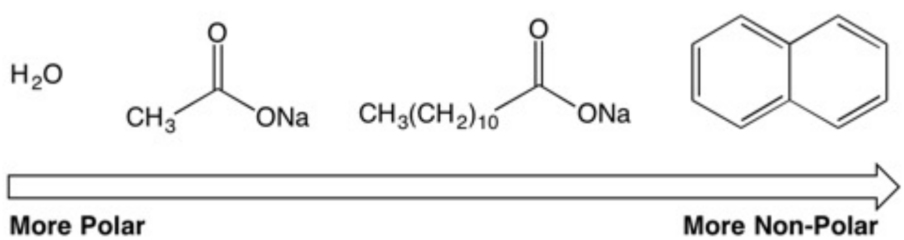


Figure 1. Examples of organic molecules that differ in polarity. Water is much more polar than sodium acetate. Sodium dodecanoate is much more non-polar than sodium acetate, but more polar than naphthalene, which is conversely much more non-polar than the other molecules.

In today's lab we will learn the art of extraction by isolating caffeine from tea. Caffeine belongs to a class of important molecules called alkaloids. These molecules are plant-derived, contain a basic nitrogen, often have a bitter taste, and commonly have physiological and/or psychological activity. Examples of some nitrogen-based alkaloids are shown on the following page in Figure 2.

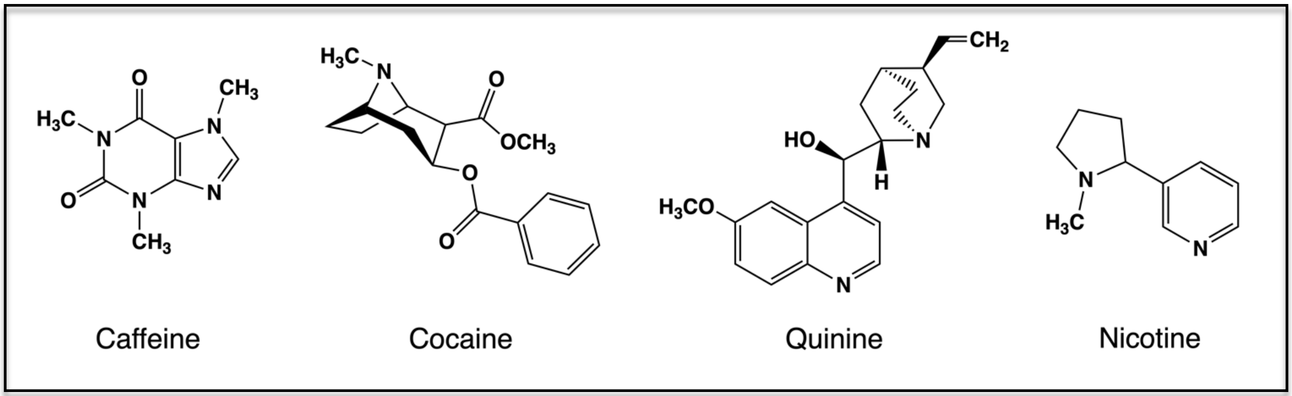


Figure 2. Examples of naturally occurring alkaloids.

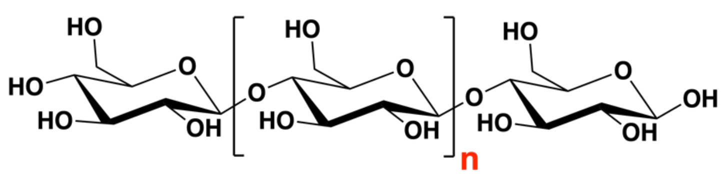
The challenge of today's experiment is to separate the caffeine from the other components of tea (Fig. 3):

1. **Cellulose:** the major structural component of plants, including tea leaves. A high molecular weight, linear polysaccharide made up of thousands of glucose units (**n** = # of glucose units in Fig 3). **Cellulose is insoluble in both water and most organic solvents**.

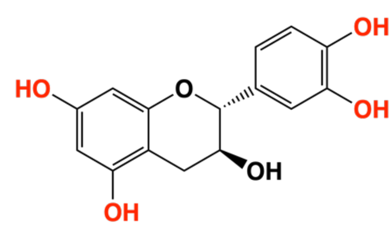
2. **Caffeine:** present in dry tea leaves at approximately 50 mg per gram of tea (concentrations vary). **Caffeine is soluble in hot water and dichloromethane (CH_2Cl_2).**

3. **Catechin / tannins**: organic molecules responsible for the color of black tea. These molecules are polyphenols that have four ArOH groups that are moderately acidic (pK_a ~ 10) and are very slightly soluble in CH_2Cl_2. **Phenoxide salts, however, are water soluble.**

3. **Chlorophyll:** green plant pigment that enables plants to convert carbon dioxide and water to carbohydrates and oxygen in the process called photosynthesis. Present in small amounts in black tea. **Chlorophyll is soluble in CH_2Cl_2**.



Cellulose (**n** = 2,000 to 26,000)



Catechin (phenol OH = red)

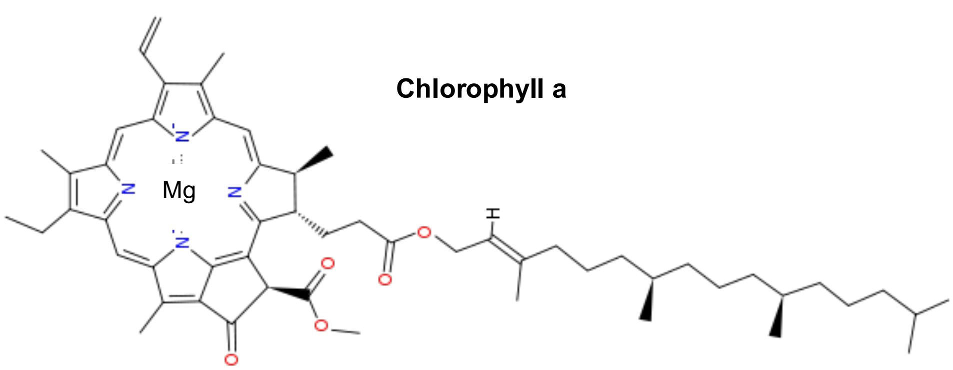


Figure 3. Other components found in tea leaves.

New Techniques

- **Extractions & Drying Agents:** See the videos posted under Lab 3 on Labflow, and Mohrig[2] for additional info.

- **Rotary Evaporator** ("Rotovap"): Your TA will demonstrate proper use of the rotovap during lab.

Chemistry

This lab's separation scheme will be more complex than the one seen in the Recrystallization lab. One of the major things to consider is the use of acids and bases in the extraction. Consider the structure of tannins (see Fig. 3). In general, they contain a phenol group (Ar-OH), which has a hydroxyl group attached to an aromatic ring. The proton on the phenol oxygen is more acidic ($pK_a \sim 10$) than a standard aliphatic alcohol ($pK_a \sim 16$). This means that it can be deprotonated by a base such as sodium carbonate, Na_2CO_3 (***this is different from sodium bicarbonate***, **$NaHCO_3$**). The practical result of this acid-base reaction is that the more non-polar tannin, which normally would only be soluble in similarly non-polar (or "organic") solvents, can be converted into its conjugate base, a *phenoxide* salt, which is soluble in water.

SAFETY ISSUES

Minimize skin contact and inhalation with dichloromethane (DCM, methylene chloride). Keep this solvent under the hood at all times and lower the sash to the appropriate level. If spilled on the skin, wash under warm water for 15 minutes. **Note:** the nitrile and latex gloves DO NOT provide protection against dichloromethane. If you spill dichloromethane on your gloves take them off immediately and wash your hands before obtaining a new pair.

Standard practices apply for the use of **gloves, safety glasses** and **pipettes** (see Lab 1).

Procedure: Extracting the caffeine (and other molecules) from tea leaves

1) Combine 100 mL of water and 5 g of sodium carbonate (Na_2CO_3) in 250-mL beaker, cover with a watch glass, and wrap snuggly with aluminum foil. Place this on your hot plate and set it to **300 °C** (the plate will reach this temperature, not the solution – the solution can only reach its boiling point!)

2) When the water begins to boil immerse **5 tea bags** into the hot water. Use your tongs to gently push the tea bags down into the water. Please be careful – steam is HOT!! Continue to heat for 10 minutes while periodically pushing the bags down into the water.

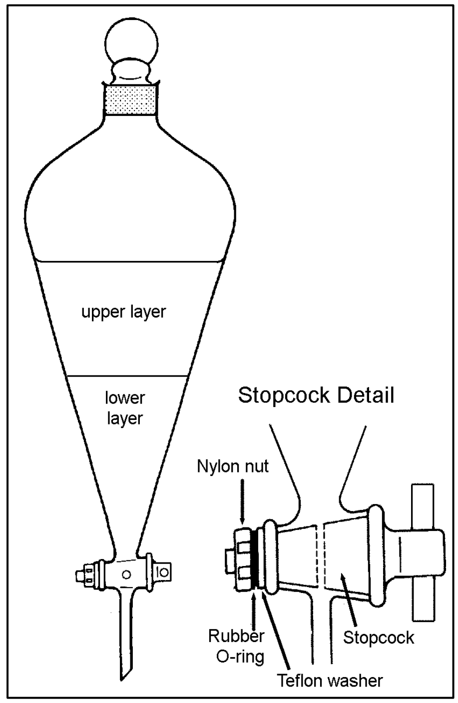
- An acid-base reaction is occurring between the tannins (general structure ArOH, where Ar is an aromatic ring) and the carbonate. **Include the balanced chemical equation in your revised separation scheme.** **(Do you expect that the organic product of this reaction will be water-soluble? Why or why not?)**

3) Carefully (steam is HOT!) remove the tea bags one at a time with the tongs and place it on a Hirsch funnel held above the beaker. Squeeze the liquid out of the tea bag by pressing GENTLY on it with a test tube. Be careful not to break the bag open. Discard the used tea bag in the regular trashcan.

4) Cool the resultant solution in an **ice-water bath** to below 40 °C (check temp with a thermometer) and pour it into the separatory funnel (be sure the stopcock is closed, use the long-neck plastic funnel to aid in the transfer). See the diagram of the separatory funnel at the top of the next page.

Note: if you notice that there are any solid particles in your solution you will have to remove them by doing a hot gravity filtration; you can use a solids-transfer filter plugged with cotton. Ask your TA for assistance.

Separate the caffeine from the other water-soluble molecules.



5) To the separatory funnel add approximately 25 mL of CH_2Cl_2, stopper the funnel and tilt it up and down **GENTLY. DO NOT SHAKE THE FUNNEL VIGOROUSLY - THIS WILL LEAD TO AN EMULSION.**

- If an emulsion does form, try stirring gently with a glass rod to break up the emulsion. Saturated NaCl solution may be required to break up the emulsion - ask your TA for assistance!

With the lower exit spout pointing at an upward angle and the stopper secured by hand, vent the sep funnel by turning the stopcock **SLOWLY** until fully open. You will hear the hiss of gas escaping and see the liquid sputter from the port. ***(What is the gas that is being released?)***

Place the separatory funnel in a ring clamp **and remove the stopper** to allow the layers to separate completely for 2-3 minutes. After the mixture has settled you should see two layers form. (What is the composition of each layer? Comparison of which physical property of water and CH_2Cl_2 will allow you to predict which layer is on top and which is on the bottom?)

6) Separate the layers by draining off the lower layer into a clean Erlenmeyer flask. Leave behind any emulsion that may have formed.

7) Extract the aqueous layer with **two** additional 20-mL portions of CH_2Cl_2 and combine the CH_2Cl_2 (organic) layers. You now should have two solutions – combined organic extracts and the aqueous solution (which contains any emulsion that may have formed). Be sure you clearly indicate the contents of each layer on your separation scheme.

You will continue to work with the combined organic layers. It is best to place your aqueous solution in the back of your hood for now. Do not throw this away until you are certain you no longer need it!

8) **Remove water from the organic layer** by drying with anhydrous sodium sulfate (a common drying agent). Add the Na_2SO_4 in small portions (~1 g per 25 mL) and swirl the flask carefully and observe how the Na_2SO_4 moves. When the sodium sulfate remains granular, as opposed to clumping, enough has been added; your solution should also be completely transparent with no haziness. Otherwise, water may still be present. Let the solution sit for 5 minutes before moving to Step 9.

9) Separate the drying agent from the organic layer. Vacuum filter the mixture, then transfer into a clean, DRY, and pre-weighed round-bottom flask. Rinse with 5 -10 mL CH_2Cl_2 to aid in the transfer of the solution. CHOOSE YOUR RB FLASK CAREFULLY – the flask should not be filled more than half-full. (*Which molecules are now in the flask at this stage?*)

10) **Remove the solvent using the rotary evaporator**. Your TA will demonstrate how to use the rotary evaporator during lab and will also operate the instrument for you. Once the rotary evaporation is complete, check to make sure your crude product is completely dry, and not damp or clumpy. If it appears damp, you may have water present. Confirm with your TA that your sample is good before moving to Step 11.

11) **Weigh the flask to determine the amount of crude caffeine you have isolated.**

12) **Using a small amount of CRUDE caffeine, obtain the crude melting point**.

You will not be purifying your caffeine sample. Instead, add a small amount (~ 1 mL) of CH_2Cl_2 to your RBF with the crude caffeine, dissolve the sample completely, and use a glass pipette to transfer the sample to a clean test tube.

13) Cover the test tube with a small piece of parafilm and label it with the following information: Initials, section number, date, name of product (caffeine)**. Store the vial/tube in the area designated by your TA. You will analyze your crude product at the next lab meeting.**

14) Clean and dry the separatory funnel and teflon stopper and return them to the drawers in the back of the lab (or place them on the drying rack above the sink). **These pieces are NOT to be stored in your bin under the hood. Be sure that the stopper is stored NEXT TO (not in) the funnel**.

Prelab Assignment

- **Prereading & Videos** - Be sure to read any documents (Labflow) or assigned pages pertaining to new techniques and chemistry in this lab (see the first page). Also check Lab 3 on Labflow for videos.
- **Pre-lab Quiz** - **On your own**, complete the pre-lab quiz on Labflow prior to your lab meeting.
- In your pre-lab notebook page(s) include the structures of caffeine, catechin, and chlorophyll, and cellulose. Hypothesize WHY caffeine is extracted from tea in basic solution. What is the advantage of deprotonating the tannins in this separation?
- **Separation Scheme** - At the top of your separation scheme include **structures and labels** for caffeine, cellulose, catechin/tannins (ArOH), and chlorophyll. Your scheme should outline each of the steps required to isolate and purify the caffeine from all the other components except for chlorophyll. **Be sure to include balanced chemical equations for the reaction of the tannins with the carbonate base.**
- **Procedure** – Include a summary of the procedural steps. *The summary should have enough detail that someone who is conducting the experiment can understand the steps without referring to the original lab handout.*
- **Table of Reagents:** ***Copy the table on the next page into your pre-lab assignment***. *Fill in the table before lab with the missing values, but not the values you'll obtain in lab.*

	MW (g/mol)	d (g/mL)	mp/ bp (ºC)	solubility in H_2O	solubility in CH_2Cl_2	Amount obtained (g)	Amount obtained (mol)
Caffeine			234-236 (mp)	20 mg/mL @ 25 ºC 680 mg/mL @ 100 ºC	140 mg/mL @ 25 ºC		
Sodium carbonate			851 (mp)	300 mg/mL @ 20 ºC	N/A		
Dichloromethane			40 (bp)	13 mg/mL @ 20 ºC	N/A	N/A	N/A

In-Lab Notebook

- **Observations:** record EXACT amounts of reagents used (use table like one shown above), observations, and any changes you have made to the written procedure. This should be written directly in your notebook as you complete the lab.
- All information above should be written directly into your lab notebook. The duplicate pages will be turned in to your TA after they have signed them before you leave lab.
- All information below will be included on the Post-Lab Report Worksheet or Typed Discussion and does NOT have to be written separately in the notebook.

Post-Laboratory Report

Complete the report as directed on the next pages.

References

1. a) Pavia, D. L., Lampman, G.M., Kriz, G.S., Engel, R.G. *Introduction to Organic Laboratory Techniques;* Saunders College Publishing: New York, 1999, pp. 119-127. b) Roy, C.P., Sebahar, H.L., Kasper, A.C. Organic Chemistry Laboratory Handout, Duke University, 2005, 2008, 2013
2. Mohrig, J. R., Alberg, D. G., Hofmeister, G. E., et al. *Laboratory Techniques in Organic Chemistry*, 4th ed. W. H. Freeman & Co.: New York, NY; 2014
3. Pavia, D. L., Lampman, G.M., Kriz, G.S., Engel, R.G. *A Small Scale Approach to Organic Laboratory Techniques.* Saunders College Publishing: New York, 2009
4. Loudon, G.M.; Parise, J. *Organic Chemistry, 7th ed.* Roberts & Co. Publishers; 2021

Chemistry 201L - Post-lab Report for Extraction - Isolation of Caffeine (Lab 3)

Name: ____ TA name/Section number: _________________________

Write out your revised separation scheme. Include the names of all four tea components at the start. Be sure to include balanced chemical equations for the reaction of the tannins with the carbonate base. **A template for this specific lab's separation scheme is provided on the last page.

Complete the following in the box below.

a. Draw the reaction mechanism **using curved-arrow notation** for the deprotonation of tannins in aqueous base. Use **ArO-H** as a generic form of a tannin and use **sodium carbonate** (Na_2CO_3) as the base. Be sure to draw out the structure of Na_2CO_3 and **specify which oxygen(s) acts as the base**.

b. Balance the chemical equation and comment on the aqueous solubilities below each of the species (assume that the tannin is insoluble in water initially).

Complete the table below using the literature data* provided for Lipton Black Tea, shown in the second column. **Note that the data is for one tea bag**; adjust your calculations as necessary.

	Mass (g) per Tea Bag	Total Theoretical Mass for this lab (g)	Percent Mass by Weight (g/g dry tea) x 100%
Dry tea leaves	2.4		
Caffeine	0.0362		
Tannins	0.0348		

*Henning, S. M. ; Fajardo-Lira, Claudia ; Lee, H. W. ; et al. *Nutrition and Cancer*, **2003**, *45*(2), 226-235

ANALYSIS (include units where necessary)

Appearance of **pure** caffeine (**literature**): ____

Appearance of **crude** caffeine (**any online source**) : ____

Appearance of **crude** caffeine (**observed**): ____

Literature pure m.p. (single or range): ____

Observed **crude** m.p. range: ____

Mass of **crude** caffeine: ____

Percent Yield of Crude Caffeine (*Theoretical amount* = 5 tea bags x 0.0362 g caffeine/tea bag):

$$\frac{actual\ grams\ of\ crude\ caffeine}{theoretical\ grams\ of\ caffeine}\ x\ 100\% \ = \ \_\_\_\_\_\_\_\_\_\_$$

Labflow Discussion

Please answer the post-lab report questions as directed on Labflow.

Also see the last page of this handout for the separation scheme template to help you lay out your ideas before submitting your post-lab report.