

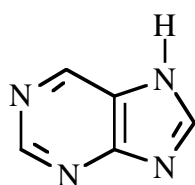
## LABORATORY 6

### Natural Product Chemistry: Isolation of Caffeine from Tea

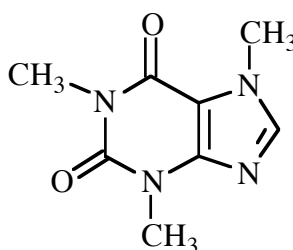
#### Introduction

Natural products are organic compounds produced by living plant and animal biochemistry. These compounds vary greatly in molecular size and chemical composition. Many have a pronounced effect on human behavior and health. For example, most antibiotics were originally extracted from microorganisms found in soils.

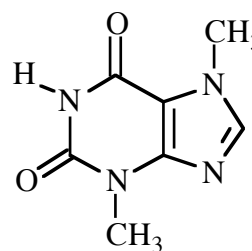
Caffeine is one of the many pharmacologically active materials found in coffee, tea, and other plants. It is perhaps the most widely used "legal" stimulant, rivaled only by its sister molecule, theobromine, which is found in the cacao bean (and chocolate). Both of these molecules have the parent purine ring structure, which is also seen in guanine and adenine (found in DNA and RNA).



Purine



Caffeine



Theobromine

In this laboratory, caffeine will be isolated from tea leaves. The techniques you will be using are familiar. Each has been explored in a separate experiment earlier in the semester. You will be revisiting extraction, recrystallization, the rotary evaporator, and thin layer chromatography. Our goal is to combine the skills you have already learned in an experiment, which mimics those actually performed by researchers in academic and pharmaceutical laboratories.

#### Reading and Pre-Lab Assignments

Read the following sections in *The Organic Chem Lab Survival Manual: A Student's Guide to Techniques* by James W. Zubrick, John Wiley & Sons, Inc.

- Drying Agents
- Extraction and Washing
- Crystallization
- Thin layer chromatography
- Using the handbooks, look up the data you will need for this experiment, for example melting points and solubilities.

Before you come to the laboratory, do the Pre-Lab assignments for this laboratory as assigned by your instructor.

Prepare your laboratory notebook as required by your instructor.

### Procedure:

In this experiment, you will carry out the following steps. We have elaborated the science behind each step involved for your clearer understanding of the operations.

**Use hot water to extract the caffeine from the tea leaves.** The solubility of caffeine is 22 mg/mL (25 °C) and 670 mg/mL (100 °C). While cellulose is insoluble in the water, the tannins and chlorophyll will extract along with the caffeine into the water.

**Use an organic solvent to extract the caffeine and related compounds from the water.** The tendency of a solute (in this case caffeine) to dissolve preferentially in one solvent over another can be represented in a **partition coefficient**. A partition coefficient is the ratio of solubilities of a compound between two different solvents, or  $P_{(\text{Solvent 1/Solvent 2})} = [\text{Solute in Solvent 1}]/[\text{Solute in Solvent 2}]$ . The partition coefficient between ethyl acetate and water is approximately 2, since caffeine is (46 mg/mL)/(22 mg/mL) or 2.1 times more soluble in ethyl acetate than water while the partition coefficient for dichloromethane is about 8 (the solubility of caffeine in dichloromethane is 182 mg/ml). If you wanted to calculate how many grams of caffeine were extracted into a solvent, you would solve for X in the following:

$$\text{Partition Coefficient} = (X \text{ g caff./vol solvent}) / ((\text{total g caff.} - X \text{ g caff.}) / \text{vol H}_2\text{O})$$

In this experiment, as with the previous Extraction experiment, you will extract with several portions of a solvent in order to increase the yield of caffeine. By knowing the partition coefficient, it is possible to mathematically prove that these multiple extractions are more efficient than a single extraction.

If relative solubility were the only consideration, dichloromethane would clearly be the better choice of extraction solvent. However, dichloromethane poses a greater health risk to both humans and the environment than does ethyl acetate. Therefore, the greener choice is ethyl acetate. Choosing to use ethyl acetate as the extraction solvent will decrease the amount of caffeine recovered but generate less hazardous waste. In this particular situation, this is a tradeoff worth making.

**Wash the solution with a base to remove the acidic tannins from your organic phase.** This works by deprotonating the acidic tannins in your solution, making them more ionic and therefore more easily dissolved in a water layer. This will be done with more than one washing for the same reason as above.

**Dry caffeine with a drying agent.** This step removes any small amount of water in your ethyl acetate.

**Remove ethyl acetate from caffeine using a rotary evaporator.** The lower boiling point of ethyl acetate relative to water lends itself to being removed with a rotary evaporator (this is like doing a distillation).

**Recrystallize the crude caffeine from hexane and acetone.** This step separates the caffeine from any chlorophyll and any other remaining impurities.

**Determine a % recovery for the purified caffeine.** Compare this value with the estimated value in tea leaves based on the assumption that 2% of the mass is caffeine.

**Test the purity of your extracted caffeine using TLC.**

### **Extraction of Caffeine into water and then into ethyl acetate**

Place 120 mL of water in a 250 mL beaker and place it on a hot plate set to high. Heat the water to boiling. Carefully remove the beaker from the hot plate and place six tea bags into the hot water. Swirl the bags in the water for two minutes. Remove the tea bags one at a time from the solution, carefully pressing the bags between two watch glasses so that the expressed liquid is returned to the beaker. Add 0.60 g sodium carbonate to the hot liquid and cool the tea solution to room temperature using an ice bath.

Pour the cooled tea solution into the separatory funnel supported in an iron ring stand. The tea must be at room temperature before the addition of the organic solvent. Add 20 mL of the ethyl acetate and gently rock the solution in the separatory funnel for at least one minute. Be careful not to shake the solution as this will cause emulsions to form. Allow the layers to separate. If you have any emulsions try breaking them up at the interface with a stirring rod. Drain the aqueous and organic layers into separate flasks. Pour the aqueous layer back into the separatory funnel and repeat the extraction with three additional 20 mL portions of ethyl acetate, combining all of the organic washes into a single flask.

Once the caffeine has been extracted into the ethyl acetate layer, the aqueous layer can be discarded. Transfer the combined organic extracts back into the separatory funnel and wash twice with 10 mL of saturated aqueous sodium carbonate. Drain the ethyl acetate layer into a clean, dry Erlenmeyer flask and add about 2-3 g of anhydrous sodium sulfate. Swirl occasionally until the sodium sulfate no longer clumps; this should take about 10 minutes.

While the solution is drying, place the waste material in the proper container in the waste hood, rinsing the beaker and the separatory funnel with a few mL of acetone. The glassware can now be safely washed in the sink.

## Isolation of caffeine

Decant the dried ethyl acetate extract into a tared, dry 100 ml round bottom flask. Place the flask on the rotary evaporator in the hood and remove all of the ethyl acetate. The caffeine should appear as a crusty deposit on the sides of the flask.

Obtain the mass of the crude caffeine. If enough solid has been isolated, recrystallize to further purify the caffeine as described below.

Using 2-4 mL portions of acetone, dissolve and wash out the caffeine from the round bottomed flask into a 50 mL flask. Place a wooden boiling stick into the flask containing the acetone solution, and boil gently on the hotplate in the hood to remove the acetone. Remove the flask from the hotplate as soon as the acetone has evaporated. Add 2 mL of hexane to the flask and 0.5 mL of acetone. Heat until the caffeine is barely dissolved. Allow the solution to cool, and then place in an ice bath. Collect the caffeine on the Hirsch funnel. Determine the mass of the extracted caffeine and report it as a % by weight of the dry tea leaves. Your instructor will tell you the mass of the tea in six bags for comparison.

## Thin Layer Chromatography of Extracted Caffeine

Since caffeine sublimates near its very high (235-237 °C) melting point, we will not use melting point to confirm the identity or assess the purity of our natural product. Recall that thin layer chromatography can separate mixtures. Recall also that we can identify substances by comparison with the  $R_f$  values of known materials. You will run a TLC of a known caffeine standard and a sample of the caffeine you extracted.

Spot 1: Spot your crude caffeine sample after dissolving it in acetone but prior to any recrystallization.

Spot 2: Place a few crystals of your caffeine product on a watch glass. Add several drops of ethyl acetate to dissolve the crystals. Dip a capillary tube into this extract and place a spot of the mixture on a TLC plate.

Spot 3: Place a few crystals of pure caffeine product on a watch glass. Add several drops of ethyl acetate to dissolve the crystals. Dip a capillary tube into this sample and place a spot on the TLC plate.

Develop the TLC plate in a mixture of 3 mL of ethyl acetate and 1 mL of methanol. Visualize the spots by short wave UV light. Calculate and compare the  $R_f$  values of the compounds.

## Results, Discussion and Conclusion

Write your results, discussion of results and your conclusion. Complete any post-lab questions.

## Abstract

This part should be filled in after the completion of the experiment and analysis of all data. When submitting the report, the abstract should appear at the beginning of the report.

## Report

Adhere to the format required by your instructor and submit the report on time.

### Summary

1. Know how different components separate themselves between aqueous and organic phases.
2. Know the principle of extraction, theory of partition coefficient and TLC.
3. Know how components move on a TLC plate and given a TLC diagram. You should be able to identify the different components on the basis of their polarities.

## Questions

1. Prepare a flowchart for the experiment. Remember to include appropriate structures and explicitly state what compounds are present and in which layer/fraction for each step.
2. What percent recovery could be expected if 1.000 g of caffeine was initially dissolved in 120 mL of water and then extracted with a single 80 mL portion of ethyl acetate?
3. What percent recovery could be expected if 1.000 g of caffeine was initially dissolved in 120 mL of water and then extracted as per the protocol given in the lab manual?
4. Percent recovery and percent yield are occasionally used as if they are interchangeable terms. They do, however, have different meanings. Why is it more correct to use percent recovery here?
5. Would the apparent percent recovery be higher, lower, or unchanged if a student did the following (answers must provide an explanation founded in the underlying chemistry):
  - a. Used room temperature water to brew the tea?
  - b. Did not add sodium sulfate when directed?