# Lab #1 – Separation and Purification of Natural Products

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## Introduction

In organic chemistry, there are many important techniques that are necessary to understand and perform. Extraction, thin layer chromatography (TLC) and sublimation are examples of these techniques and they will be reviewed in this lab in order to purify caffeine from tea.

For scientists, it is very important to obtain the purest chemical compound as possible in order to obtain the best results. Extraction provides a way of purifying chemicals based on the solubilities of the compounds involved with two immiscible solvents. Compounds with high water solubility will be in the aqueous solvent, while those with lower water solubility (usually organic compounds) will show up in the organic phase with the organic solvent. These two layers can then easily be separated using an extraction funnel. The extraction process should be carried through multiple times in order to obtain the best results.

Recrystallization by sublimation is another technique used to further purify compounds and is carried out using an apparatus called “cold finger”. As a side-arm flask containing a crude solid and a test tube filled with ice is heated in a vacuum, the molecules vapourize and condense as crystals on the “cold finger”. The idea in this process is to purify the molecule by forcing it to sublime and condense on the cold-finger, leaving the impurities behind.

Finally, thin layer chromatography is used to verify that the compound is purified. TLC analyzes the difference in polarities of the components of the mixture. The mixture is dissolved in a mobile phase and is passed over a stationary phase. The different components of the mixture will stick either to the mobile phase or stationary phase based on the differences in polarity. Typically, the stationary phase is polar, while the mobile phase is non-polar. If a component is polar, it will interact strongly with the stationary phase and thus will not travel very far. On the other hand, a non-polar molecule will not interact well with the stationary phase and will migrate faster and farther. The sample mixture and the reference mixture are both spotted on the same TLC plate to compare the polarities of the components in the respective mixtures. If the spots line up perfectly, the sample and reference mixture are the same.

Using the techniques mentioned, it is possible to isolate caffeine directly from brewed tea. As the tea brews in the hot water, caffeine and other molecules enter in solution in the water. Due to their non-polar characteristics, caffeine and tannins are soluble in organic solvents, unlike the polyphenols which remain in the aqueous phase. To isolate caffeine in the organic phase, an acid base reaction should be conducted to deprotonate the tannins and make them more soluble in water. By performing multiple extractions using an organic solvent (such as dichloromethane), the caffeine can be isolated in the organic phase, while the others stay in the aqueous layer. By evaporating the organic solvent, the caffeine will solidify and can be further purified using sublimation. TLC analysis will provide insight on the actual purity of the final product. The TLC of the crude product compared to the reference caffeine sample theoretically will not match up perfectly due to the fact that impurities still remain. The recrystallized version and the reference caffeine, however, should theoretically be made up of the same components and match up perfectly.

**Table of Reagents**

Table 1 – Table of Reagents and Solvents

| Compound | Molar Mass (g/mol) | Quantity (g or mL) | Density(g/mL) | Mmol |
|---|---|---|---|---|
| Sodium Carbonate | 105.99 | 2.00 g | 2.532 | 18.9 |
| Dichloromethane | 84.93 | 44 mL | 1.325 | 690 |
| Distilled water | 18.02 | 60 mL | 1.00 | 3300 |
| $Na_2SO_4$ | 142.04 | 2.21 g | 2.66 | 15.6 |
| NaCl | 58.44 | 5.00 mL | 2.16 | 185 |

**Experimental Procedure**

Refer to CHM2123 Lab Manual pg. 28-30

**Observations and Results**

Table 2 – Table of Observations

| Key Step | Observation |
|---|---|
| Addition of Tea Bags to Boiling Water | - Water turns dark brown as the tea steeps<br>- Tea steeps for about 15 mins |
| Addition of $Na_2CO_3$ to Steeped Tea | - $Na_2CO_3$ is a white powder<br>- Powder sticks to the bottom a little but dissolves eventually |
| Extraction with dichloromethane | - Aqueous layer (dark brown) is on top; organic layer (clear) on bottom<br>- Residue remains in the beaker containing the steeped tea<br>- An additional 14 mL of dichloromethane is added due to poor separation of the layers<br>- During funnel shaking, a small amount of liquid leaked from the top.<br>- 5mL of NaCl (brine) added in final extraction<br>- Extracted organic layer is colourless but cloudy with a ring of brown on the top<br>- Extracted aqueous layer is a very dark brown liquid. |
| Addition of $Na_2SO_4$ to organic phase | - Brown and white clumps form at the bottom; liquid on top is clear. |
| Steam Bath | - Solid crude caffeine is off-white, flaky powder<br>- Smells like caffeine |

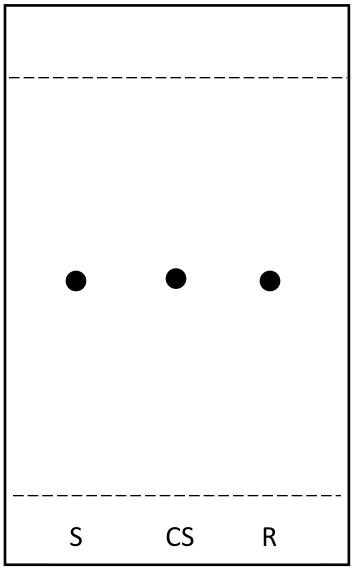
| | |
|---|---|
| | - Product remains stuck on the sides of the flask |
| Sublimation | - Crude product was left on the hotplate for a while before dry ice was able to be added therefore all product was lost. |
| TLC | - Crude caffeine solution went missing, therefore solid crude was dissolved in dichloromethane in order to spot the TLC<br>- The TLC for the recrystallized caffeine was not able to be done because the product was lost. |

Table 3 – Table of Results

| **Compound** | **Mol. Mass (g/mol)** | **Quantity (g or mL)** | **Mmol** | **Yield (%)** |
|---|---|---|---|---|
| Empty Tea Bag(2) | - | 0.120g x 2 = 0.240g | - | - |
| Tea + Bags (2) | - | 7.04 g | - | - |
| Tea | - | 7.04-0.240 = 6.80g | - | - |
| Crude Caffeine | 194.19 | 0.03g | 0.154 | 11.0 |

**<u>TLC Plates</u>**

99:1 acetone:acetic acid



$R_F$= 0.59

Sample (S): Crude Caffeine
Cospot (CS): Crude Caffeine + Authentic Caffeine
Reference (R): Authentic Caffeine

The TLC Plate for the Recrystallized Caffeine was not able to be completed due to the loss of all of the product. Theoretically, the recrystallized caffeine and the authentic caffeine should have the same characteristics and therefore should line up on the TLC plate. That being said, their $R_F$ values would be the same.

## Calculations

1. Percent Yield

Theoretical value = 6.80 g x 4% = 6.80g x 0.04 = 0.272 g
Actual value = 0.03g

$$\% \text{ Yield } = \frac{actual\ value}{theoretical\ value} \times 100\%$$

$$= \frac{0.03g}{0.272g} \times 100\%$$

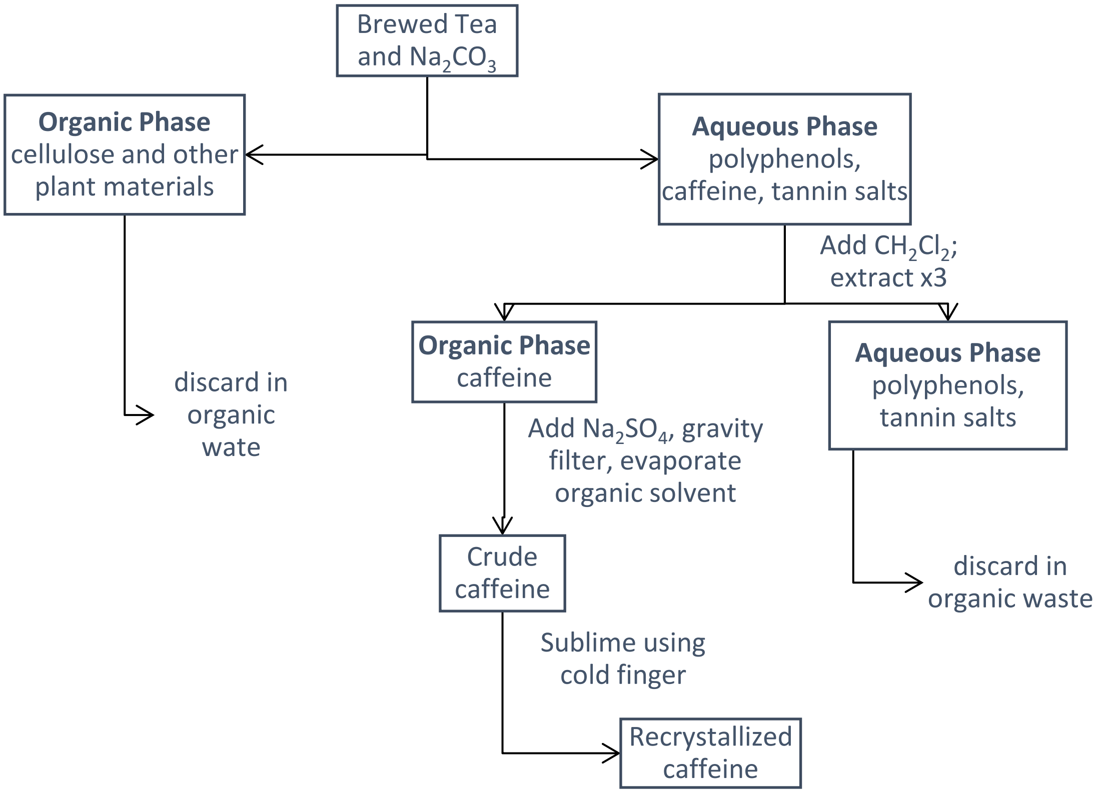
$$= 11.0\ \%$$

2. $R_F$ Value

Distance travelled by compound = 3.2 cm
Distance travelled by solvent = 5.4 cm

$$R_F = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}} = \frac{3.2}{5.4} = 0.59$$

## Flow Chart



## **Discussion**

The goal of this experiment was to isolate and purify caffeine from brewed tea using extraction, sublimation and then confirming with TLC analysis. The tea was brewed in boiling water for 15 minutes in order to allow as much caffeine to enter in solution with the water as possible. Next, $Na_2CO_3$ was added to the steeped tea to conduct an acid-base reaction so that the components of the tea could be separated into organic and aqueous layers. The sodium carbonate deprotonates the tannins, which lead to a difference in polarity of the components, thus different solubilities. Dichloromethane was added to the separatory funnel as the organic solvent where the caffeine is dissolved; all other compounds from the tea are dissolved in the aqueous layer. The aqueous layer was found to be on the top, while the organic layer was on the bottom due to its higher density. During the experiment, a small amount of brown emulsions appeared so brine was added to eliminate them. NaCl increases the ionic strength of the aqueous layer, which helps to push the organic material out of the aqueous layer; this ensures proper separation of the phases.

After several extractions with dichloromethane, a drying agent ($Na_2SO_4$) was added to the organic layer to remove any water that may be present in the organic phase of the extraction. This created brown and white crystals that remained at the bottom of the flask. These solids were removed using gravity filtration, and the liquid was placed in a steam bath in order to evaporate the solvent and isolate the caffeine. The product was an off-white, flaky powder and smelled a little like coffee, therefore it is known that this product was indeed caffeine. This was confirmed during the TLC analysis of the crude product compared with the sample of authentic caffeine. The spots lined up perfectly and both solutions had the same $R_F$ values, which proves that the solid was caffeine and that the crude product was relatively pure.

Unfortunately, the experiment did not proceed further due to the complete loss of the product. During the sublimation process, the crude caffeine was added to the flask and placed on the hotplate for too long before any dry ice could be added to the cold finger. This caused the product to evaporate quickly resulting in the loss of all the product. A cold-finger apparatus is used to force a solid directly into the gaseous phase without becoming a liquid. The caffeine is heated in a vacuum such that it sublimes and condenses on the cold-finger, leaving the impurities behind. Theoretically, the recrystallized form of caffeine would no longer contain any impurities. That being said, the mass of the purified caffeine would be less than the crude caffeine because it no longer contains the impurities that added to the mass. The TLC of the purified caffeine would have only one spot and it would line up perfectly with the reference authentic caffeine. The $R_F$ values of the authentic caffeine and recrystallized caffeine would be the exact same.

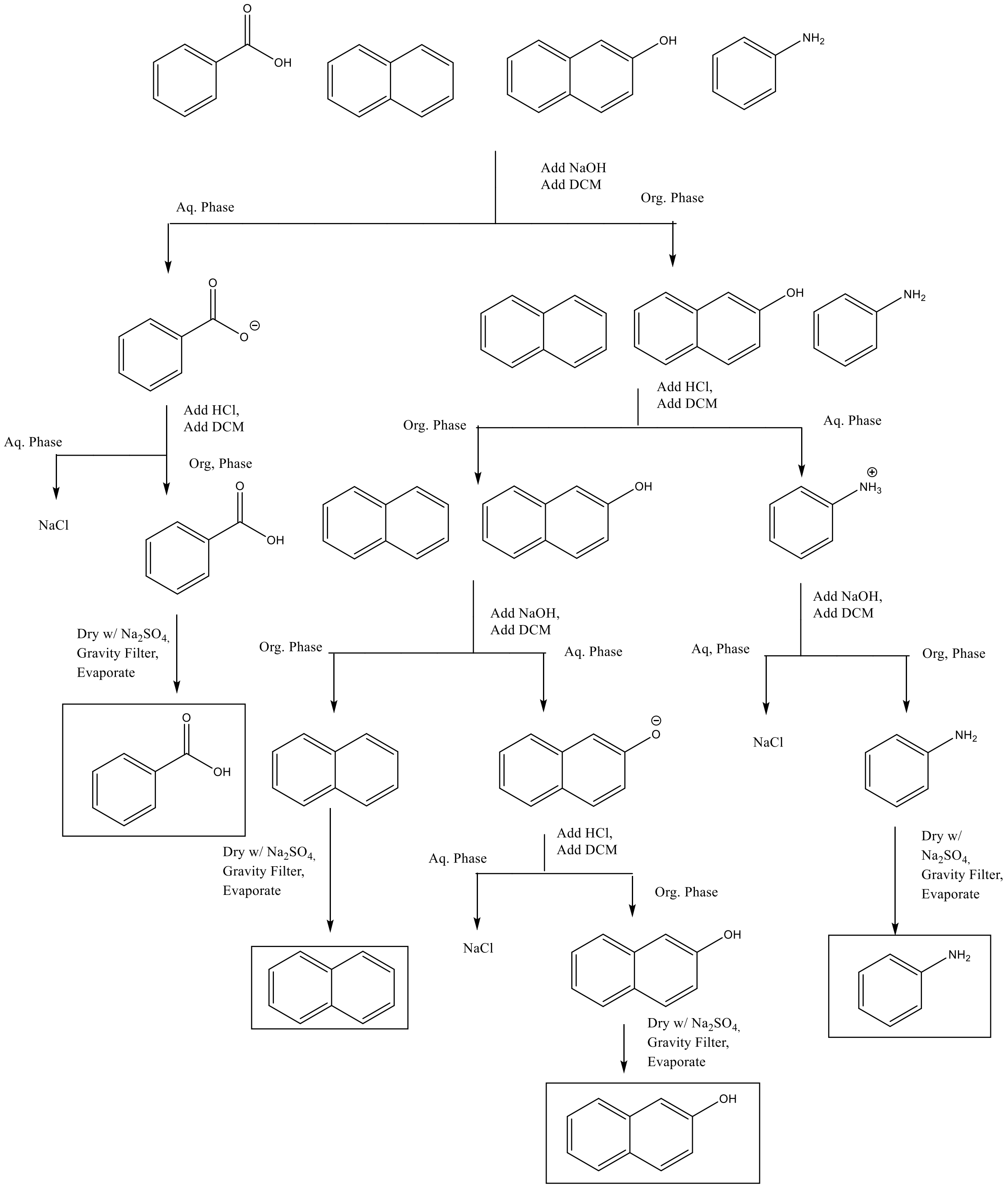
The mass of tea that was brewed in water was 6.80g and the mass of crude product obtained was 0.03g. Assuming the tea contained 4% caffeine by weight, the percent yield of caffeine is 11.0%. Since the sublimation process could not be complete, the crude product was considered to be the final product and was used to determine this percent yield. The reason why this yield was so low is because small amounts of product were lost consistently throughout the experiment. First, when the tea bags were removed from the water, it is assumed that a large amount of caffeine did not enter in solution and was thus discarded with the tea bags. During the extraction, a small amount of liquid leaked from the top and it is also possible that the organic phase was not separated from the aqueous layer completely, resulting in the loss of product. Crude product remained stuck to the sides of the flask after the solvent was evaporated and the product was scrapped out.

Although this yield is quite low, the yield of the recrystallized caffeine would be significantly lower, assuming that the crude caffeine was not 100% pure. The impurities add to the mass, which leads to a higher percent yield calculation.

**Questions**

1. The NaCl (brine) solution functions to remedy an emulsion by helping the two layers separate properly. NaCl will dissolve in the solution and increase the ionic strength of the aqueous layer, therefore the organic and aqueous phase cannot interact.
2. Catechin is a polar polyphenol, therefore it is soluble in water. To extract this molecule from brewed tea using a liquid-liquid extraction, an organic solution with lower density would have to be added to the flask. This would force the aqueous layer to be at the bottom, and should be extracted multiple times using water. At this point, the crude product is in solution in the aqueous phase, therefore it would have to be further extracted with a drying agent and suction filtration.

3.



4. A pencil should be used to mark a TLC plate as opposed to a pen because the ink could be moved up the plate and therefore affect the TLC results. This occurs because the mobile phase dissolves the ink thus the ink will migrate up the plate, causing splotches.