# Lab #1 – Separation and Purification of Natural Products

**Andrea F.**

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CHM2123

## Introduction

This lab uses the techniques of extraction, thin layer chromatography (TLC) and sublimation to extract caffeine from tea. Furthermore, extraction and TLC were used to identify the individual pigments contained in spinach leaves.

The type of extraction used in this lab is called the liquid-liquid extraction, which involves two immiscible liquids. The two liquids have to be immiscible, in order form two separate layers, or phases, when mixed together. The two phases that form in such an extraction are called the organic and the aqueous phases. The organic phase consists of an organic solvent and the aqueous phase is mainly comprised of water. The compounds contained in the solution will be extracted into one of these layers depending on their polarity and density, as some of them will be more soluble in the organic, and other in the aqueous phase. The desired compound will be moved from one phase to the other and impurities are left behind in the initial phase.

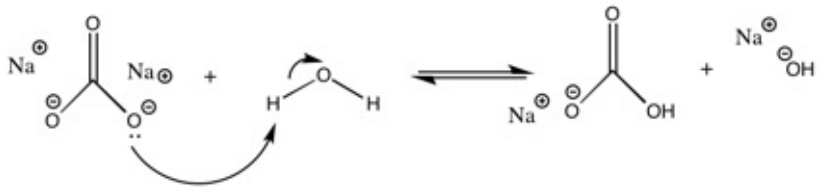
TLC is another way of separating the components of substances, and consists of a stationary phase, here a thin layer of silica gel on an aluminum plate, and a mobile phase, a suitable liquid solvent. Silica gel contains a fluorescent substance which glows under UV light, enabling the visualization of the results.

In this experiment, the solvents used were a mixture of 99:1 acetone:acetic acid for part A, and a mixture of 50:25:25 cyclohexane:hexane:acetone for part B.

Sublimation is a technique in which a solid substance is vapourized and the vapors are then condensed to form a solid again without going through an intermediate state. To achieve this, a cold sleeve was used in this experiment, which is made up of a test tube filled with dry ice, in this case, placed in a side-arm flask containing the isolated solid crude product. The flask is heated, causing molecules to vapourize and condense in form of small crystals on the surface of the cold finger. The isolated purified product can then be scraped off the side of the cold finger and used for further purposes.

## Mechanism

Figure 1: Reaction mechanism for the acid-base reaction between $Na_2CO_3$ and water.



## Table of Reagents and Solvents

Part A

| Reagent | Molecular Mass (g/mol) | Quantity | Density (g/ml) | Mmol | Equivalents |
|---|---|---|---|---|---|
| **Distilled $H_2O$** | 18.02 | 60.0 ml | 1.0 | 3329.63 | |
| **$Na_2CO_3$** | 105.99 | 2.04 g | 2.54 | 19.25 | |
| **Dichloromethane** | 84.93 | 45.0 ml | 1.33 | 529.85 | |
| **$Na_2SO_4$** | 142.04 | 1.52 g | 2.66 | 10.70 | |
| **Tea** | - | 6.46 g | - | - | |
| **Caffeine in tea** | 194.19 | 0.2584 g[1] | 1.23 | 6.33 | |

Part B

| Reagent | Molecular Mass (g/mol) | Quantity | Density (g/ml) | Mmol | Equivalents |
|---|---|---|---|---|---|
| **Spinach leaves** | - | 1.0 g | - | - | |
| **Anhydrous $Na_2SO_4$** | 142.04 | 1.0 g | 2.66 | 7.04 | |
| **Acetone** | 58.08 | 4 ml | 0.791 | 68.87 | |
| **Hexane** | 86.18 | 3 ml | 0.659 | 34.81 | |
| **$H_2O$** | 18.02 | 3 ml | 1.0 | 166.48 | |

## Experimental Procedures

Refer to CHM2123 lab manual, pages 23-34.

### *Modifications*

Part A

- Did not require to do step 8, as the emulsions in the separatory funnel cleared up and two separate layers formed after the additional 10 ml of dichloromethane were added.
- The melting point of the purified crystals was not measured as we did not have enough time left in the session.

Part B

- Step 8 was repeated and two TLC plates were developed instead of one, using the solvent mixture of 3:7 hexane:acetone for the second TLC plate, instead of 50:25:25 cyclohexae:hexane:acetone

[1] We assume that the tea contained 4% caffeine by weight, meaning that we have 0.2584 g of caffeine contained in the 6.46 g of tea.

## Observations

Part A

| Key Step | Observations |
|---|---|
| $Na_2CO_3$ added to tea after teabags were removed | - $Na_2CO_3$ is a white powder<br>- turned tea a darker brown and more opaque<br>- (tea was a lighter brown and clearer before the addition) |
| Added dichloromethane to solution in separatory funnel | - layers did not separate at once<br>- a single brown layer with brown "bubbles" at the bottom formed during the first extraction<br>- brown "bubbles" were contaminations in the organic layer caused by the aqueous layer |
| Added another 10 ml of dichloromethane to the separatory funnel in order to induce the separation of the two phases | - the two layers separate very slowly<br>- swirling the separatory funnel instead of shaking it speeds separation up a little bit (shaking mixes the two phases again)<br>- the aqueous phase is the brown layer on the top<br>- the organic phase is the clear, colourless layer on the bottom<br>- caffeine is in organic phase, as it is more soluble in dichloromethane |
| Added $Na_2SO_4$ to the organic layer | - $Na_2SO_4$, a white powder, is a drying agent which removes excess water from the organic phase<br>- Formed small chunks of solids at the bottom of the beaker as it bound the water |

Part B

| Key Step | Observations |
|---|---|
| Crushed spinach leaves, sand and $Na_2SO_4$, and added acetone | - $Na_2SO_4$ is a white powder<br>- acetone is a clear, strong smelling liquid<br>- solid spinach pulp settled at the bottom of the tube, darker green liquid on top of spinach leaf layer |
| Preparing test tube A | - single layer of opaque dark green liquid |
| Preparing test tube B, added hexanes to the solution from test tube A | - two separate layers form<br>- the aqueous layer is the opaque light green layer on the bottom<br>- the organic layer is the dark green layer on the top<br>- the organic layer contains the chlorophyll, as chlorophyll is more soluble in dichloromethane than in water<br>- the higher concentration of chlorophyll in the organic phase causes the organic phase to have a darker green colour |

| | |
|---|---|
| Preparing test tube C, added HCl to the organic phase from test tube B | - two layers form<br>- the organic phase is the opaque light green layer on the top<br>- the aqueous layer is the clear, transparent layer on the bottom |

## Table of Results

Part A

| Compound | Molecular Mass (g/mol) | Quantity | Mmol | Yield (%) | Melting Point (°C) |
|---|---|---|---|---|---|
| **Crude caffeine product** | 194.19 | 0.02 g | 0.103 | - | - |
| **Purified caffeine crystals** | 194.19 | 2.19 g | 11.28 | 178.20 | 239.90[2] |

Part B

| $R_F$ value | TLC colour/description | | |
|---|---|---|---|
| | **A** | **B** | **C** |
| **0.02** | brown | - | - |
| **0.39** | yellow-green | yellow-green | - |
| **0.43** | green | green | - |
| **0.69** | - | - | blue-green |
| **0.88** | green | green | yellow-green |

[2] Theoretical value taken from CHM2123[A], Exp 1 - report + data, Brightspace

## TLC Plates[3]

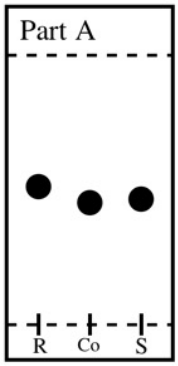



Figure 2: TLC plate #1 for the experiment (Part A).

Solvent system: 99:1 acetone:acetic acid
Reference spot: crude caffeine product
Co-spot: purified caffeine product
Sample spot: authentic caffeine

$R_f$ value for the reference lane is: 0.51
$R_f$ value for the co-spot lane is: 0.45
$R_f$ value for the sample lane is: 0.47

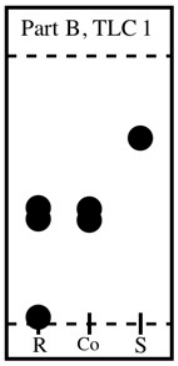



Figure 3: TLC plate #2 for the experiment (Part B).

Solvent system: 50:25:25 cyclohexane:hexane:acetone
Reference spot: content of test tube A
Co-spot: content of test tube B
Sample spot: content of test tube C

$R_f$ value for the lower spot ($A_1$) in the reference lane is: 0.02
$R_f$ value for the middle spot ($A_2$) in the reference lane is: 0.39
$R_f$ value for the upper spot ($A_3$) in the reference lane is: 0.43
$R_f$ value for the lower spot ($B_1$) in the co-spot lane is: 0.39
$R_f$ value for the upper spot ($B_2$) in the co-spot lane is: 0.43
$R_f$ value for the sample lane is: 0.69

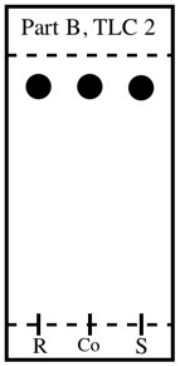



Figure 4: TLC plate #3 for the experiment (Part B).

Solvent system: 3:7 hexane:acetone
Reference spot: content of test tube A
Co-spot: content of test tube B
Sample spot: content of test tube C

$R_f$ value for the reference lane is: 0.88
$R_f$ value for the co-spot lane is: 0.88
$R_f$ value for the sample lane is: 0.88

[3] For all TLC plates (from left to right): Reference lane, co-spot lane, sample lane.

## Calculations

Sample mole calculation:

$$n(caffeine\ crystals) = \frac{mass\ of\ caffeine}{molecular\ mass\ of\ caffeine} = \frac{2.19\ g}{194.19\ {}^{g}/_{mol}}\ x\ \frac{1000\ mmol}{1\ mol}$$

$$n(caffeine\ crystals) = 11.28\ mmol$$

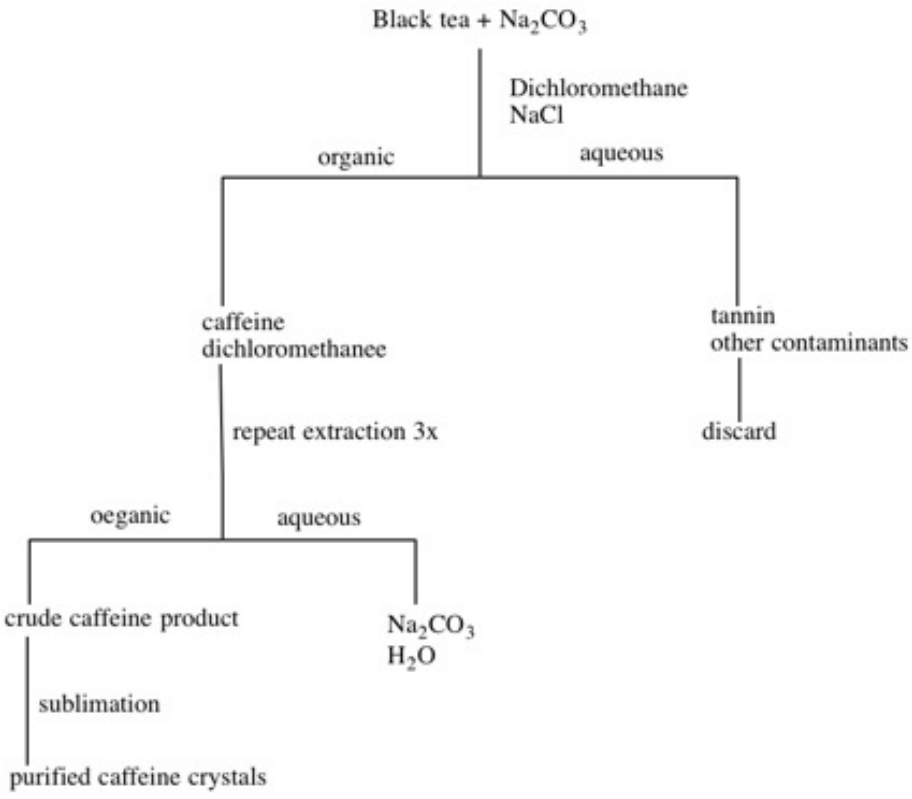
Calculating the percent yield of caffeine:

$$\%yield = \frac{mol\ product}{mol\ starting\ material}\ x\ 100\% = \frac{11.28\ mmol}{6.33\ mmol} x\ 100\%$$

$$\%yield = 178.20\%$$

## Flow Diagram

Part A




## Discussion

The purpose of this experiment was to review extraction, TLC and sublimation, all of which are frequently used techniques to isolate and purify the product obtained from a chemical reaction. All three of these

techniques were used in part A of the experiment, where the goal was to isolate caffeine from black tea. Part B required extraction and TLC in order to observe the different pigments contained in spinach leaves. For part A, $Na_2CO_3$ was added to strong black tea and the solution was then extracted three times with dichloromethane. The solution did not separate into the separate aqueous and organic phases at once, forming a single brown layer with clear bubbles throughout the bottom of the solution. A further 10 mL of dichloromethane were added to the solution to induce the extraction and the solution was swirled around the separatory funnel instead of shaken, as shaking it seemed to mix the two layers together again. The two layers separated slowly, with the brown aqueous phase being on top and the clear organic phase containing the caffeine on the bottom. The caffeine ends up in the organic phase as it is more soluble in dichloromethane than it is in water, which makes up most of the aqueous phase.

$Na_2SO_4$, a drying agent, was added to the organic phase in order to remove the excess water from the organic phase, which was then filtered so that the solids comprised of water and the drying agent that formed at the bottom of the solution could be removed. The crude caffeine product was obtained by using a steam bath in order to evaporate the rest of the organic phase. The mass of crude caffeine product obtained was 0.02 g.

In order to purify the crude caffeine product, sublimation was performed and the mass of purified crystals obtained was 2.19 g, giving a 178.20% yield. This should not be the case, as the percent yield for a properly performed experiment should be under 100%. An error which might have led to this result is that ice was accidentally scraped off the cold finger along with the purified caffeine product, leading to an increase in mass and inaccurate results for the mass of purified product. Another error could have been the loss of crude product due to too much suction during the sublimation process.

After the purified product was obtained, TLC was performed to compare the crude and the purified products to authentic caffeine. From the TLC plate, it can be observed that the purified caffeine product ($R_f$ = 0.45) has a very similar $R_f$ value to the authentic caffeine ($R_f$ = 0.47) and that the crude caffeine product has a higher $R_f$ value at 0.51. This shows that the contaminants contained in the crude product were successfully removed during sublimation.

Since the time given for the experiment was not sufficient, the melting point of the purified product was not determined. However, the theoretical value for the melting point of the purified caffeine product is 239.90°C[4].

In part B, the different pigments contained in spinach leaves were observed using extraction and TLC. In order to separate the pigments from the spinach leaves, liquid-liquid mini extraction was used, where the leaves were torn into small pieces and were crushed in a test tube with sand and anhydrous $Na_2SO_4$. This enabled the removal of water and pigment from the leaves, and a layer of dark green liquid formed on top of the crushed plant material which settled at the bottom of the test tube. 2 mL of acetone were then added and the tube was shaken in order to mix the contents and was left to settle after. This was repeated twice more. The dark green liquid was then collected into test tube A and an additional 2 mL of acetone were added to the solution to increase its volume.

Half of the contents of test tube A were then transferred to test tube B, and hexanes were added. A mini extraction was performed by shaking the test tube and the solution separated into two layers, with a dark green organic layer on top and a lighter green aqueous layer on the bottom. The organic layer has a darker green colour as chlorophyll is more soluble in dichloromethane than in water, and the organic phase is mostly made up of dichloromethane. Half of the resulting organic layer was transferred to test tube C, where 1 mL of HCl was added to the solution and another mini extraction was performed. The solution separated, forming two separate layers where the light green organic layer was on top and the clear aqueous layer on the bottom.

[4] Theoretical value taken from CHM2123[A], Exp 1 - report + data, Brightspace.

Next, the separated pigments were visualized using TLC. TLC was performed twice, once with a solvent system of 50:25:25 cyclohexane:hexane:acetone and the second time with a solvent system of 3:7 hexane:acetone. For both TLC plates, the solution in test tube A was spotted onto the leftmost lane, the organic layer from test tube B onto the middle lane, and the organic layer from test tube C on the rightmost lane.

Multiple dots can be observed in each of the lanes on the first TLC plate, indicating the different pigments contained in spinach leaves. It can also be concluded that the more extractions are performed, the more impurities were removed from the organic layer. This is shown by the leftmost lane, spotted with the content of test tube A (no extraction performed), containing the most dots, namely three, after being developed. The three dots had the Rf values of 0.02, 0.39 and 0.43. The middle lane, containing the organic layer from test tube B (one mini extraction performed), showed two dots with $R_f$ values of 0.39 and 0.43. The rightmost lane which was spotted with the organic layer from test tube C (two mini extractions performed) only showed one dot with the $R_f$ value or 0.69.

The dots with $R_f = 0.39$ had a yellow-green colour, indicating the pigment xanthophyll, while the dots with $R_f = 0.43$ were green, indicating chlorophyll *a*. The single dot in the rightmost lane was blue-green, indicating the pigment chlorophyll *b*. Finally, the dot in the leftmost lane with $R_f = 0.02$ showed an impurity. From the $R_f$ values we can conclude that the most polar pigment is xanthophyll. Chlorophyll *a* is slightly less polar than xanthophyll, and chlorophyll *b* is the least polar as it has the largest $R_f$ value.

The results for the second TLC plate do not show helpful results, as all three dots have $R_f = 0.88$, indicating that the solvent system was too polar. This means that the compounds rose too quickly and the results are thus unreliable. The solvent system used for the first TLC plate for this part of the experiment was less polar, meaning that the compounds rose more slowly, giving more accurate results.

Error sources for part B could have been not crushing the spinach leaves enough, thus not collecting a sufficient amount of pigments. Another error source would have been using too little or too much solvent when preparing the contents of the three test tubes, this would either result in the pigment being too diluted or not having enough solvent. Either way it would lead to unreliable TLC results. Unreliable TLC results can also be caused by spotting too much or too little solution onto the TLC plates. Spotting too much solution onto a TLC plate could cause the dots to blur and spotting too little solution would lead to very faint dots or dots the rise too quickly. Both errors would give incorrect TLC results.