University of Ottawa

CHM212

Laboratory of Organic Chemistry II

Section B00

Exp. 1: Separation and Purification of Natural Products

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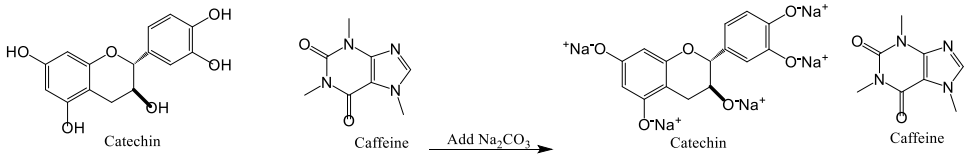
Introduction

The purpose of this experiment is to effectively separate and purify products from natural compounds through important techniques such as extractions, thin layer chromatography (TLC) and sublimation. In Part A, caffeine will be separated from black tea leaves using an acid-base extraction and purified via cold finger sublimation, while in Part B pigments will be separated from spinach leaves using liquid-liquid extractions. TLCs will be explored in both parts to compare the respective final products.

Extractions are a common technique used in organic chemistry laboratories to separate a mixture into two immiscible phases: organic phase and aqueous phase. Considering the solubility and polarity of each compound, a desired compound can be isolated and dissolved in one phase while leaving the remaining impurities to dissolve in the other phase. In Part B of the experiment, this type of liquid-liquid extraction will be performed to separate pigments of spinach leaves into different phases based on the compounds densities and solubility in each solvent. Furthermore, acid-base chemistry can be applied to extractions to protonate or deprotonate organic compounds and force them into the aqueous phase. Following separation, the newly charged ion can be protonated or deprotonated as necessary to return it to its former state. This can be favourable when separating organic compounds from one another, as will be done in Part A of the experiment to separate polyphenols from caffeine:



Through the deprotonation of the polyphenols, the polyphenols can be separated into the polar aqueous phase and the caffeine can be isolated in the organic neutral phase.

Recrystallization is a useful technique for purifying a final product, and will also be explored in this experiment through cold finger sublimation. Sublimation is the process during which a gas changes to its solid state, without changing to its liquid state in the process. This type of recrystallization is favourable for compounds that are highly volatile, have a high vapour pressure as well as high melting point, and will be used for the purification of caffeine. The crude product will be heated in a decreased pressure environment and as it evaporates it will sublimate on a test tube filled with dry ice (cold finger). The decreased pressure will increase volatility and therefore lower the heat required for evaporation. As it sublimates on the cold finger, it will take the form of purified crystals.

A TLC analysis is a highly versatile tool in organic chemistry laboratories and can used in a variety of comparisons and analyses, as it will be in both Part A and B of this experiment. TLC analysis compares the polarity of different compounds in a mixture as they are moved through a stationary silica-gel phase using a mobile solvent phase. The distance travelled by the compound is divided by the distance travelled by the solvent, to give a ratio called the Rf value. Due to increased interaction with the polar stationary gel, a polar compound will travel shorter distances, giving it a smaller Rf, while an organic compound will have limited interaction with the gel and be able to travel further, giving it a higher Rf value. In Part A, a TLC will be performed to compare the crude and purified caffeine products to a standard sample. In Part B, two TLCs will be performed to compare and identify multiple pigments found in spinach leaves.

Finally, a melting point will be taken to test the purity of the caffeine product in Part A. Pure compounds have a smaller range in their melting point, and a trace amount of impurity in a compound will cause its

range to increase significantly. By finding the melting point of the caffeine product, the purity can be assessed based on the size of the range, as well as by how close it lies to literature values of caffeine's melting point.

Experimental Procedure

As described in the lab manual (Experiment 1: Separation and Purification of Natural Products, Katherine McGilvray, 2017, Exp. 1, p.30-34)

Modifications

-Step 9: Repeat step 8 in a 30:70 hexane:acetone solvent system

Observations and Results

**Data for Part A is borrowed from Brightspace*

Table 1.1: Substances in extraction and recrystallization of caffeine*

Substance	**Mass (g)**	**Physical observations**
Mass of tea bag (g)	6.78	White paper casing with brown loose leaves
Mass of tea (g)	6.54	Brown loose leaves
Na_2SO_4	Two scoops	White powder
Mass of crude product (g)	0.4316	White, fine powder
Mass of purified crystals (g)	0.06	White, wet powder

Table 2.1: Observations of Key Steps in Caffeine Extraction

Step	Observations
Steeping tea bags in boiling water	The clear water turned a dark brown colour
Addition of dichloromethane and caffeine mixture to separatory funnel	The two phases separated with the dark brown aqueous phase settling on the top and the clear organic phase on the bottom. The presence of brown leaves stuck on the walls of the separatory funnel suggest a tear in the teabags during steeping
Addition of Na_2SO_4 to the organic layer	A formation of translucent, pale brown salts was seen settling in the beaker, which were then filtered out of solution
Evaporation of solvent	There was a residue of fine, white powder on the base and walls of the beaker after solvent evaporated
Sublimation of crystals	Crystals formed on the base of the cold finger, but quickly disappeared when a vapour began to form in the flask. A wet powder paste was left on the walls of the cold finger
TLC analysis	The recrystallized caffeine contained a compound that aligned with the compound in the standard caffeine sample

Table 2.2: Observations of extraction of Carotenoids and Chlorophylls

			TLC colour and description		
			A	B	C
Rf Value	50:25:25 cyclohexane:hexane: acetone solvent system	0.62	Grey; extremely faint	Grey	Grey
		0.55	-	Dull yellow; extremely faint	Dull yellow; saturated pigment
		0.43	-	-	Bright yellow

		0.42	Fluorescent yellow-green	Fluorescent yellow-green	-
		0.40	Dull green	Dull green	-
	30:70 hexane:acetone solvent system	0.98	Bright green single spot; highly pigmented	Bright green, single spot; highly pigmented	Bright yellow, single spot; highly pigmented

Table 3.1: Results in caffeine extraction*

Compound	Mass obtained (g)	Theoretical Yield (g)	Percent Yield (%)	Melting Point (*C)
Caffeine	0.042	0.2616	15.3	239.9

Calculations

1. Theoretical yield

Tea contains 4% caffeine by mass, therefore:

$Theoretical\ yield = 4\% \times Initial\ mass\ of\ tea$

$Theoretical\ yield = 0.04\ \times 6.54g$

$Theoretical\ yield = 0.2616g$

2. Percent Yield

$$Percent\ yield = \frac{Actual\ yield}{Theoretical\ yield} \times 100\%$$

$$Percent\ yield = \frac{0.04g}{0.2616g} \times 100\%$$

$Percent\ yield = 15.3\%$

3. Rf Values: Sample calculation for standard caffeine in TLC Plate 1.0

$R_f = \frac{d_{substance}}{d_{solvent}} = \frac{3.4cm}{5.2cm} = 0.55$

TLC Plates

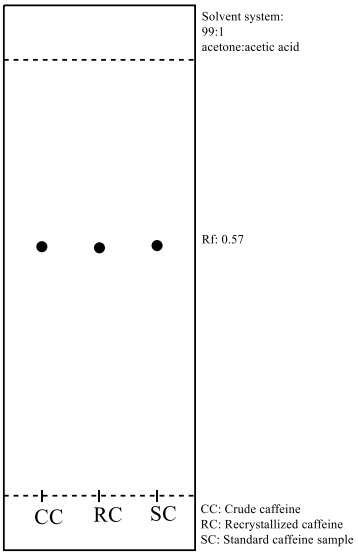


Figure 1: TLC Plate 1, comparing crude, recrystallized and standard caffeine in a 99:1 acetone:acetic acid solvent system (*Data borrowed from Brightspace)*

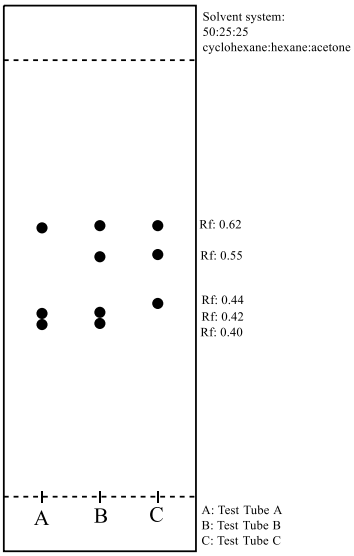


Figure 2: TLC Plate 2, comparing pigments in Test Tubes A, B, and C in a 50:25:25 cyclohexane:hexane:acetone solvent system

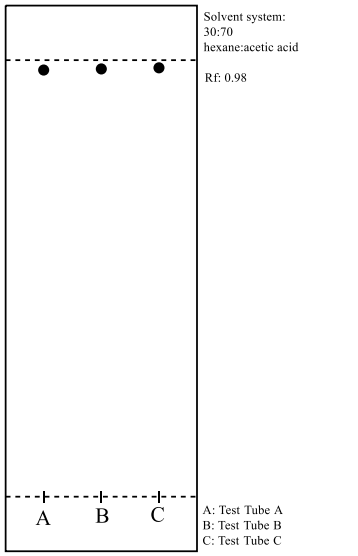
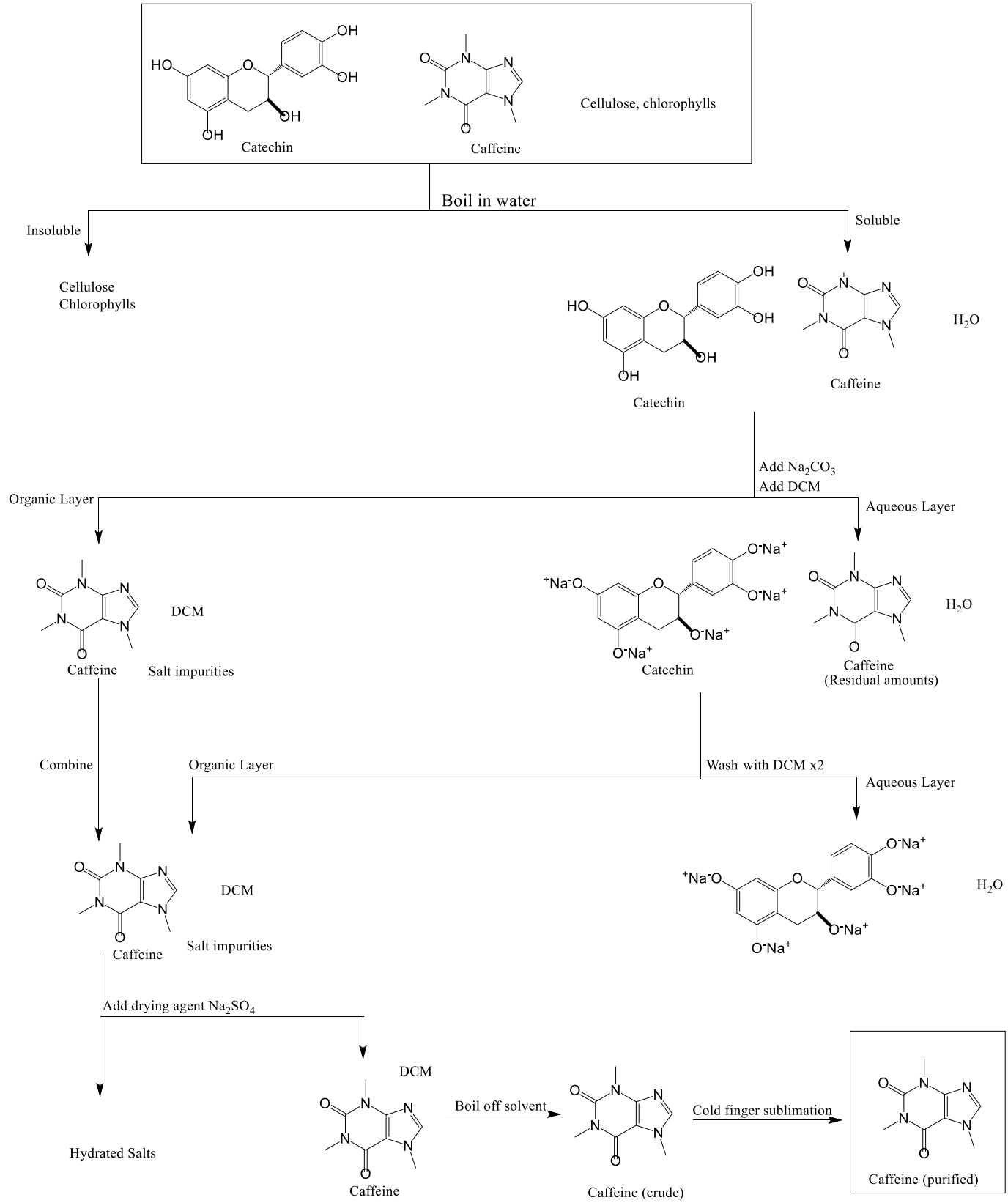


Figure 3: TLC Plate 3, comparing pigments in Test Tubes A, B, and C in a 30:70 hexane:acetone solvent system

Flowchart: Extraction of caffeine from black tea leaves



Discussion(12)

The purpose in Part A of this experiment was to extract caffeine from black tea leaves and purify the product. Following the steps outlined in the flowchart above, the first step was to boil the tea in water to dissolve the caffeine and polyphenols in water. The insoluble matter, such as the chlorophylls, cellulose and tea bag were separated from the organic compounds and discarded.

With the organic compounds isolated, the next step was to extract the caffeine from the remaining polyphenol impurities. As both major compounds in the mixture were organic, an acid base extraction was needed to separate them. Sodium carbonate, a strong base, was added to deprotonate the polyphenols and form an ionic species that became soluble in the aqueous phase. The caffeine was resistant to deprotonation due to its higher basicity, and therefore remained soluble in the organic phase. A dichloromethane (DCM) solvent was used for the extraction, which is denser than water and therefore resulted in the organic layer with the caffeine settling at the bottom of the separatory flask. As the extraction was performed, traces of tea leave residue were stuck on the walls of the flask, suggesting a potential source of error. This residue caused a blockage in the flask opening due to its higher density than the DCM and complicated the extraction of the organic layer by introducing impurities into the organic layer. This may be prevented in future experiments through filtering the tea solution prior to extraction. Following the extraction, which included two washes with DCM to ensure complete extraction of the organic compounds, the organic layers were combined and sodium sulfate was added. The sodium sulfate acted as a drying agent to remove any residual water in the organic layer, the effects of which were seen when pale-brown, hydrated salts began to form in the flask. These salts were filtered out of the solution and discarded, leaving an extracted organic mixture of DCM and caffeine. Finally, to finish the extraction of the crude product, the DCM solvent was boiled off in a steam bath to leave only crude caffeine. In the experiment, the crude caffeine was observed as white, fine powder along the walls of the beaker, indicating success of isolation.

The following step in Part A was the purification of the crude product, which was completed using cold finger sublimation. The crude product was collected and placed in a clean, dry flask with a reduced pressure. Once placed on a heating plate, the cold finger (also clean and dry) was filled with dry ice and inserted in the flask. The environment's reduced pressure consequently increased the caffeine's volatility, which resulted in a lower temperature needed to evaporate the solvent. As the caffeine evaporated, it sublimated on the cold finger and took a crystal form. Crystals began to form, however shortly after, water vapour was introduced in the environment and created a steam that wet the crystals. Instead of taking a crystal form, the purified product resembled a white paste, which was a major source of error. The water vapour may have been introduced from vapour in the air due to lack of airtight environment. To avoid this error in future experiments, proper equipment should be used with an airtight seal that will produce proper conditions for recrystallization. Due to the introduction of water vapour, the final product did not resemble a crystal form, but rather a wet paste. Furthermore, massing the final product revealed that its mass was larger than the mass of crude product, which confirmed that the recrystallization was unsuccessful and contaminated with water. Finally, a TLC supported suspicions of error, as the recrystallized product did not align with the standard caffeine sample. This experiment demonstrated the importance of maintaining a completely dry and clean environment for a cold finger sublimation, as data may be rendered unusable in the final steps. In order to complete the analysis, data was referenced from another group.

The final step in Part A was to assess the purity of the final product. As mentioned above, a TLC was performed to compare the recrystallized product to a standard caffeine sample, as well as to see if any other compounds were present. The referenced TLC displayed the final product aligning with the standard

caffeine with no other compounds present in the mixture, which suggest the crystals were pure caffeine. Furthermore, the melting point of the final product was determined to be 239.9 °C, which lies close to the literature value of caffeine's melting point: 238 °C. It is unclear from the referenced data whether a range was taken for the melting point, but the proximity to the theoretical value suggests once more that product obtained is pure caffeine.

In Part B of this experiment, extraction and TLC were used once more to compare pigments in spinach leaves. The pigments found in spinach are chlorophylls and carotenoids, two nonpolar compounds that are soluble in the organic layer. To begin isolating the pigments from other insoluble compounds, a sample of spinach leaves was ground with sand to break down the cellulose. Sodium sulfate was used as a drying agent again in order to remove water stored in the leaves. Acetone was added to this fine pulp and the nonpolar pigments were able to dissolve in this organic layer. After the sample was shaken and the organic layer containing the pigments settled atop the pulp, it was able to be extracted with a pipette and transferred to Test Tube A. This organic phase was a single dark green layer and contained the chlorophylls present in the spinach, two of which were clearly seen in the TLC analysis: one with an Rf value of 0.42 and one with an Rf value of 0.40. The former was a fluorescent green, while the latter was a duller green shade, which both contributed to the coloration of the leaves.

This original organic layer was then sampled into Test Tube B, where hexanes and water were added and shaken to perform a mini-extraction. The hexanes and water separated into two layers: a dense, yellow aqueous layer and a less dense, green organic layer. Hexanes are a strong organic solvent and were able to dissolve the organic pigments from Test Tube A, while the water was able to dissolve vitamins and polar components. A TLC analysis showed the same results as from Test Tube A: two green pigments with Rf values of 0.42 and 0.40. Another spot became clearly seen (while it was only faintly seen before): a grey, more polar spot with an Rf value 0.62. The introduction of a polar, aqueous layer may have allowed for more polar compounds to dissociate into ions and therefore travel through the silica gel than in the predominantly organic Test Tube A.

Finally, the organic phase from Test Tube B was transferred to Test Tube C and diluted with 1.0M HCl to form two layers: one green-yellow on top and one clear at the bottom. The denser clear layer was the organic hexanes, while the green-yellow layer was the organic layer containing the reduced pigments. In the addition of HCl, the chlorophylls were reduced with 2H+ to remove the Mg^{2+} ion from the chlorophyll and form new pheophytin pigments. A TLC analysis of Test Tube C revealed newly visible yellow spots and an absence of the previous green spots. The yellow spots had Rf values of 0.44 and 0.55, with the 0.44 being a duller yellow and the 0.55 being a brighter yellow colour. The grey polar spot with Rf value of 0.62 was also visible.

Analyzing the TLC plates with this given information, the bright green (Rf=0.42) and dull green (Rf=0.40) spots can be concluded as the chlorophyll pigments. They are green in colour and no longer visible when the reduction occurs in Test Tube C. The dull yellow (Rf=0.44) and the bright yellow (Rf=0.55) spots can be concluded as the xanthophyll, polar pigments that contribute to the coloration of spinach. They are visible once reduction occurs and the richer colour of the chlorophyll is gone. They are and have a higher polarity than the chlorophyll and pheophytin, which gives them a lower Rf. Finally, the grey spot (Rf=0.62) can be concluded as pheophytin pigment found in the spinach leaves. Although they are present in more than one environment, they are less polar than xanthophyll and therefore must have a higher Rf. A possibility for their faint presence before acid-reduction in Test Tube C is that prior to the initial extraction, a small percentage of the chlorophyll was already reduced. This may have occurred

multiple ways, for instance if the spinach leaf used in the sample was aging and beginning to rot, thereby naturally reducing its chlorophyll to pheophytin over time.

The solvents used to develop the TLC plates impacted how many compounds were observable. In the 50:25:25 cyclohexane:hexane:acetone solvent, the mixture was mostly nonpolar and therefore had minimal interaction with the silica gel. Nonpolar compounds are not able to interact in hydrogen bonding with the gel the same way polar compounds can, due to nonpolar compounds' lack of hydrogen donors or acceptors. This solvent dissolves nonpolar compounds and allows them both to travel faster through the TLC, yielding higher Rf values for nonpolar compounds. The polar compounds, however, experience hydrogen bonding with the gel and move far slower, yielding lower Rf values. This was seen in TLC Plate 2. In a 30:70 hexane:acetone system, the opposite effect was observed. The polar solvent was able to dissolve polar species spotted, and allowed them to move with the solvent at a higher rate through the gel. This yielded in much larger Rf values for polar compounds. TLC Plate 3 displays all polar pigments moved at the same rate as the solvent and all had an Rf value close to 1, making it unfavorable for observing the different species in the lanes. In order to optimally observe species in a solution, the proper solvent must be chosen for expected species.

Reference

Data for Part A analysis cited from Brightspace (*Dr. Katherine McGilvray, Brightspace Announcement, 22/09/2017*)