Separation and purification of natural products:

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Due date: 29th Sept 2017

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Introduction:

Extraction, Thin-layer chromatography (TLC) and sublimation are all highly important method that are used in Organic chemistry that help to isolate desired compounds from large mixtures, as well as to purify these compounds. Using Thin-layer chromatography to check for impurities once the experiment is either on going or once it has been completed.

Extraction was used in part A of the experiment to remove the Caffeine from the tea mixture that includes cellulose, chlorophyll, polyphenols and tannins. Extraction was through a variety of methods including heat, solvents of polarity and using Dichloromethane to separate Caffeine from the polyphenols. Meaning the organic layer of the solution was underneath the aqueous layer.
Sublimation allowed the crude caffeine to be heated straight to a vapour before quickly condensing to caffeine crystals through the use of the cold finger. This removed any solvent that was left in the solid caffeine. These caffeine crystals were then used further in Thin-layer chromatography.
Thin-layer chromatography then allowed the purification of the solution to be determined, due to there being different compounds in tea, if there were any components other than caffeine left in the crystals they would move up the TLC differently due to their different polarities.

Extraction was also used in Part B of Experiment 1, to remove the pigments from the spinach leaves. Then to determine which pigments were present a Thin-layer chromatography was used, due to the different polarities of the pigments and affinities for the solvent, they will move faster and further up the silica gel. Which allows the pigments to be separated and seen either by eye or under UV. The different Rf values and colours obtained from the TLC allowed the pigment types to be determined.

Mechanism:

Drying agent (Na_2SO_4) removing water:

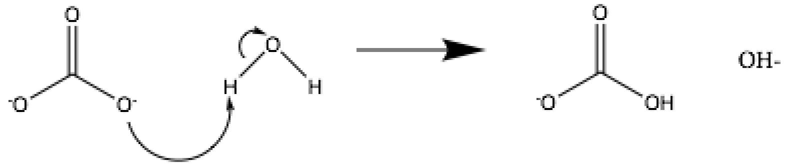


Table of reagents and solvents:

Part A of Experiment

Name	Amount
Water	60 ml
Tea bags	2 - 6.78 g
Na_2CO_3	2 g
Dichloromethane	25 ml (10ml 1st & 5 ml & 10 ml if in turbid phase)
Saturated NaCl	15 g
99.1 Acetone : acetic acid	10 ml

Part B of experiment:

Name:	Amount:
Spinach leaves	1-2
Na_2SO_4	1 g
Sand	1 g
Acetone	2 ml
Hexane	3 ml
Water	3 ml

1.0M HCL	1 ml

Experimental procedure:

Refer to CHM2123 lab manual for the experimental procedure.

Part A: pages 30, 31 and 32

However Adaptations were made to the following steps :

- In step 2, the tea bags were measures, with the weight for 2 tea bags being 6.78g
- Step 7, the funnel was shaken rapidly to form a layer of aqueous phase then formed on the bottom of the separation funnel, but it needed to be the organic phase on the bottom of the funnel so it could be removed. Therefore more dichloromethane was added to distinctly separate the layers, allowing the organic phase to move to the bottom
- Step 8 was not performed as it was not deemed to be necessary
- Step 11, 3 scoops of Na_2SO_3, the drying agent, was used to generate crystals of salts and remove all the excess water
- Step 12, a solid separation funnel should of been used to collect the solid salts
- Step 15, the caffeine crystals that were contained was 0.03g
- Step 21: the side arm flask was not put directly on the hot plate, but rather 1cm above using a clamp to prevent burning the crude caffeine
- Step 24, the sublimation was not complete since there was still some crude caffeine left in the side-arm flask but ran out of time in lab, the TLC would not of been able to be completed to check for impurities
- Step 26 was not performed due to low yield of the sublimation procedure
- Step 28 and 29, a test tube was not used instead a small 50 ml beaker was used as it was made difficult to access the solution in the test tubes using a capillary tube.
- Step 30 - a larger spotted sample was added to the TLC plate in each lane to observe the results more clearly

Part B: pages 32 and 33

Adaptations were made to the following steps:

- Step 2, an alternative stirring rod was used due to the thickness of the teflon-rod would not allow mixture of the sand that was clumped at the bottom of the centrifuge tube.
- Step 7 - a more larger spotted sample was added to the TLC plate in each lane to observe the results more clearly
- Step 8 - there was an additional experiment done where the same liquids from A, B and C were used but the solvent was changed to 30:70 hexane: acetone, this was done

because one of the solvents has a lower polarity, allowing the pigments to move further up the silica gel as it moves faster.

Results and observations:

The total yield of Part A of the experiment to extract the crude caffeine product was determined as follows:

Yield: % yield = $\frac{0.01}{6.78} \times 100\% = 0.147\%$

Part A:

Table 1

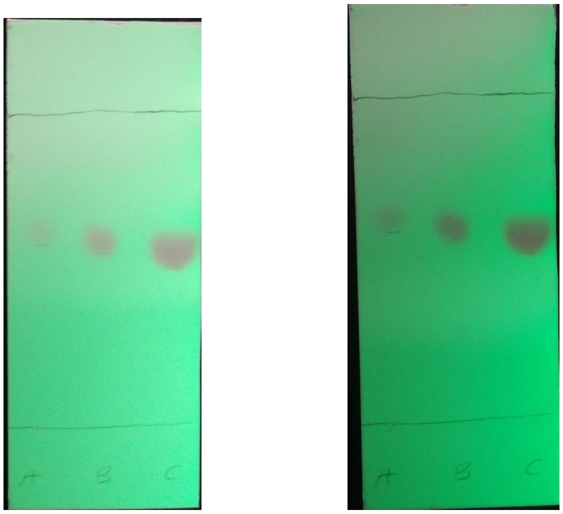
Key Step	Observations
Extraction of caffeine in organic phase	- Dichloromethane which is the organic phase was located at the bottom layer in the separatory funnel. - As the funnel was shaken faster than expected, part of the aqueous layer was moved the bottom of the separatory funnel. To remove it some more dichloromethane was added which meant that the aqueous layer moved up to the top layer, leaving a distinct separation . - During collection of organic phase, a small volume from aqueous layer also was collected unexpectedly.
Addition of drying agent (Na_2SO_4 to the organic phase)	- Pale brown salt crystals were observed at the bottom of the beaker. - The organic solution was transparent.
Boiling of the organic solvent	- When the dichloromethane was exported in the steam bath, a light yellowish powder was seen in the beaker.

Sublimation of caffeine	- The sublimation was not performed to completion leaving a lot of crude caffeine at the bottom of the erlenmeyer flask. - Colorless product was formed around the cold finger.
Thin layer chromatography	- The 3 caffeine samples were then separated using TLC, and observed under UV light. Pink dots were seen and measured.

TLC plates:

Table 2

Rf Value		TLC colour/ description		
		A- crude caffeine	B- pure caffeine	C- reference caffeine
2.7 / 4.4	0.614	Under UV - pale pink		
2.8 / 4.4	0.630		Under UV- Mid pink	
2.6 / 4.4	0.590			Under UV - mid pink



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Figure's 1 and 2:

These are TLC plates from the Caffeine extraction, which is Part A of Experiment 1
A = The sublimed caffeine
B = The crude caffeine
C = The authentic caffeine acting as the reference

Part B:

Table 3

Test Tube	Description
A	Was dark green liquid that was transparent
B	Organic phase on top - dark green that was transparent Aqueous phase - light green that was cloudy
C	Organic phase - lighter green and transparent Aqueous phase - clear in colour

TLC plates:

Table 4

Rf Value		TLC Colour		
		A	B	C
50:25:25 of cyclohexane: hexane: acetone				
0.7 / 4.1	0.171	Very pale green	Very pale green	-
1.1 / 4.1	0.268	Mid-green	Pale green	-
1.9 / 4.1	0.463	-	Extremely pale green	Extremely pale green
0.9 / 4.1	0.220	Olive green	Olive green	-
1.0 / 4.1	0.244	yellow	yellow	yellow
0.3 / 4.1	0.073	Pale yellow	Pale yellow	Very pale yellow
30:70 hexane: acetone				
0.4 / 4.5	0.080	Only seen under UV		
4.2 / 4.5	0.930		Mid-pale green	Mid-pale green

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Figure 3:

This the TLC plate form Part B of Experiment 1, the extraction of colour pigments from spinach leaves, using 30:70 hexane:acetone solvent
A = test tube A solution - CH_2Cl_2
B = test tube B solution - hexane & water
C = test tube C solution - HCl

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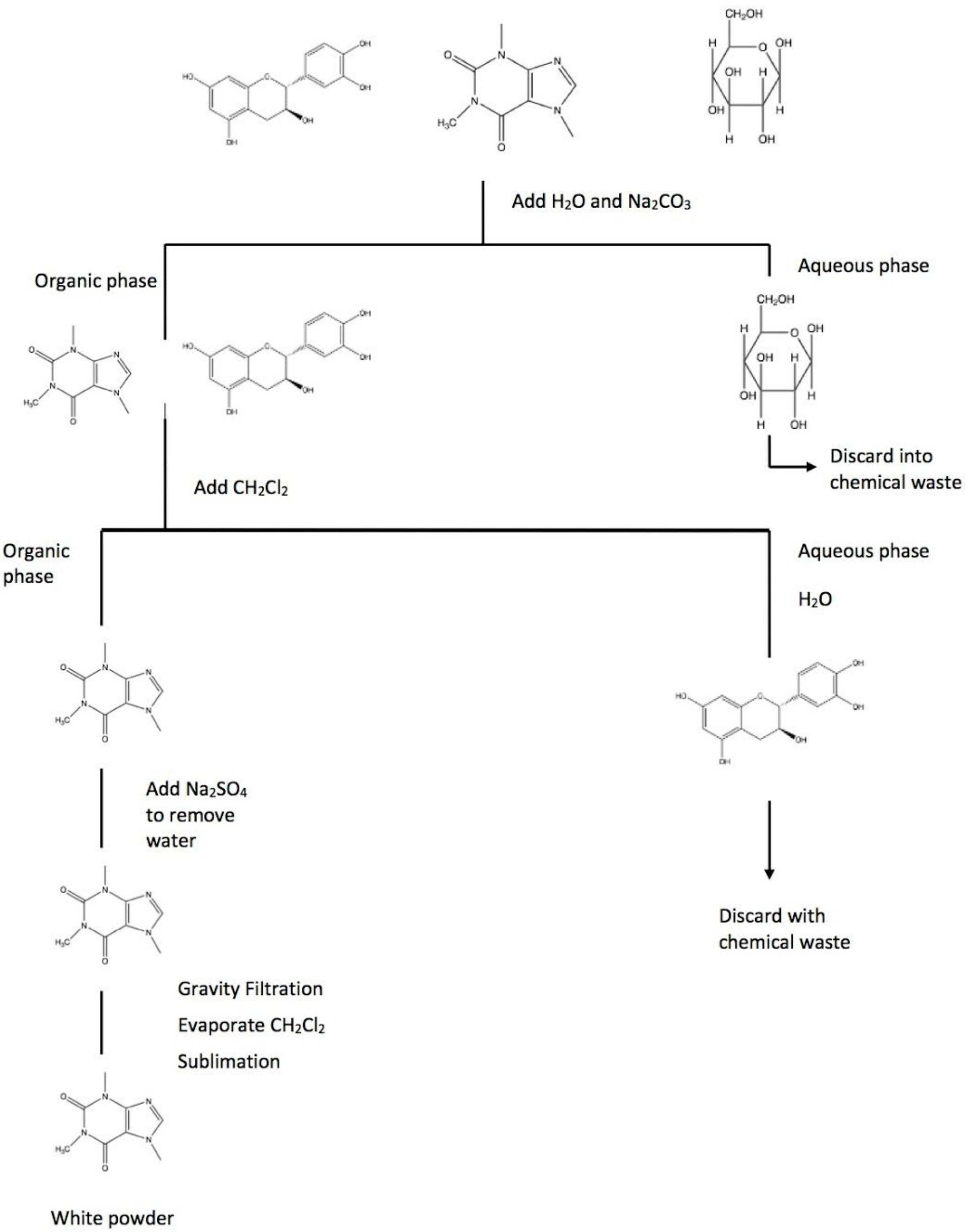
Figure’s 4 and 5

This the TLC plate form Part B of Experiment 1, the extraction of colour pigments from spinach leaves, using 50:25:25 cyclohexane:hexane:acetone solvent
A = test tube A solution - CH_2Cl_2
B = test tube B solution - hexane & water
C = test tube C solution - HCl

Flow diagram (Part A):

(notes acid, base, acid, base reactions)



Discussion:

Part A:

The main aim of Experiment 1 Part A was to obtain a purified sample of Caffeine from the samples of tea using dichloromethane as an extractor due to it's separatory qualities, as an organic solvent to remove the polyphenols that would of remained in the sample after heating. This is due to their higher affinity for water rather than the dichloromethane and could therefore be removed once the solutions have separated themselves into distinct layers by their density. With the aqueous solution being on top and the organic phase, with the desired caffeine, on the bottom it was able to be filtered out. This step can be repeated many times to ensure results and gain an end product of pure caffeine powder. With the addition of Na_2SO_4 used as a drying agent, to remove excess water. Due to it's properties as a salt. Any aqueous phases were discarded into the chemical waste to prevent harm to the environment.

To further purify the caffeine all solvent has to be removed, so the organic phase was steam bathed to remove a large quantity of solvent but not all can be removed this way. Which was why sublimation was carried out, as Caffeine has a specific melting point of between 230.2 - 236.2, whereas CH_2Cl_2 and the Na_2SO_4 have the melting points of -96.7 °C and 884 °C respectively. Therefore, the caffeine crystals would be the only compound to be annealed to the cold finger once it reached 230 °C within the erlenmeyer flask. This is also a method to help determine if a sample is pure, if it is not and other compounds are present in the powder then it would melt at a lower temperature as the bonds in the structure are not uniform.

Which was the reason for the TLC to be done after the Sublimation of the caffeine, as it was compared against a known sample of pure caffeine, to determine if there was any other compounds left in the powder. Acetic acid: acetone was the solvent because it had a good affinity to separate the caffeine from other compounds if they had been there. As the lines present for the caffeine powder and the reference caffeine were in very near concordance with each other. It can be inferred that the end caffeine product was pure. The slight difference between results in Table 2 were due to the measurements being taken from the middle of the spot on the silica gel, each of the spots were varying in size as seen in the Figure’s 1 and 2. The final yield being 0.147% was a low yield but that was due to not all of the crude caffeine being melted in the Sublimation procedure due to lack of time. However it is only estimated that tea contains 4% caffeine, therefore the comparison in expected and received results is not large.

Part B:

As for the second part of experiment 1, it's purpose was to determine the pigments that were present in spinach leaves. The initial compounds that the spinach leaves were mixed with, was

Na_2SO_4 and sand, these were to used as a drying agent and hard crystalline structures to break up the spinach and release the pigments. Acetone was further used to release the pigments to obtain a more concentrated sample. This would then in turn yield better TLC results as the subsequent samples would also be more concentrated.
Hexane and water were added to a subsequent sample to separate the aqueous and organic phases, with the organic layer being on the top due to its smaller density. This organic layer would contain the pigments that give the leaves it's colour. They would be further separated by the addition of hydrochloric acid (HCl) as it protonates any base compounds left making them ionic and therefore more water soluble. They would leave the organic phase and move to the aqueous phase, which can be removed as waste, and discarded in organic waste.

The 3 samples were then used for TLC plating, to obtain the pigments that were in the spinach leaves and could be observed. As shown from the Table 4 above there was an abundance of green and yellow colourings. This could possibly be due to chlorophyll B as it gives a green colouring in comparison to chlorophyll A that gives a blue-green colour. Furthermore due to the samples possibly being exposed to a strong acid naturally, or starting to degrade, the chlorophylls could of be converted to Pheophytins by the displacement of Magnesium and addition of hydrogen. This would in turn give the olive associated colour on in lanes A and B presented in Table 4. With the yellow pigment possibly being derived from the Xanthophylls.
The second TLC plate with 30:70 hexane:acetone was a higher polarity solvent, in comparison to 50:25:25 cyclohexane:hexane:acetone. Leaving the pigments to move further up the silica gel, however there was not as many colours observed in this solvent, only one line was in each lane observed and all being at a middle green colour from the chlorophyll A. This may be due to the other pigments having a lower affinity for this solvent.