

Isolation of Caffeine from Tea Leaves via Acid-Base Liquid-Liquid Extraction

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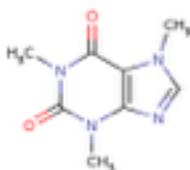
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Abstract: Caffeine was extracted from tea by the use of solid-liquid and liquid-liquid extractions. An acid/base liquid-liquid extraction took place in order to force caffeine into the organic layer. A pure product of .065 g caffeine was obtained. This gave calculated values of 59.1 % recovery and 40.9 % error. Caffeine was the final product. Many sources of error may have occurred such as an incorrect calibration of the scales with which the samples were measured and not “washing” the solution thoroughly enough to obtain as much sample as possible.

Introduction:

Tea is one of the most commonly used caffeinated beverages in the world. The caffeine ($C_8H_{10}N_4O_2$) found in tea is a bitter, white, crystalline methylxanthine and a member of a class of compounds known as alkaloids (Wang, 2011). Alkaloids are basic nitrogen containing compounds present in plants. The structure of caffeine affects the functions it performs.



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Alkaloids, such as caffeine, are often physiologically active in humans and are known central nervous system stimulants and diuretics (Wang, 2011). Caffeine also causes an increase in respiration and heart rate, as well as nervousness and insomnia. Though caffeine has demonstrated to have physical dependence, it is also capable of improving alertness, learning capacity, and exercise performance (NCBI, 2013). Tea leaves, in which caffeine is found, also contain acidic tannins, undecomposed chlorophyll, cellulose, and pigments. In order to extract caffeine from tea leaves, caffeine must be present as the free base (Amrita, 2013). In order to do so, the above-mentioned acidic substances must remain water-soluble .

In order to extract caffeine from tea, several methods are used. First, a solid/liquid extraction must take place in order to get the solid natural product into the liquid solvent. This can be done by using a Soxhlet extractor, or by simply brewing a cup of tea. In order to isolate the desired reaction compounds from the natural product, liquid/liquid extractions are used.

Neutral and acid/base are two forms of liquid/liquid extractions (Williamson, 2011) . Caffeine extraction from tea leaves involves an acid/base liquid/liquid extraction (Oneota, 2003) . The reaction involves a homogenous mixture of an organic and aqueous layer. The ideal solvent in the extraction should have a low boiling point, not react with the solute or other solvents, not be toxic or highly flammable, not miscible with water, be inexpensive, and should readily dissolve caffeine at room temperature. A common liquid/liquid solvent pair for the extraction of caffeine is water-dichloromethane (Williamson, 2011). Because water is present in the pairing, it possible to separate inorganic compounds from organic compounds due to the fact that organic substances are immiscible in water (Amrita, 2013).

When mixing the liquid pairs, the density of the both solvents predict which solvent is the top and which is the bottom layer. Caffeine, which was present in the organic layer, was located below the aqueous layer (Williamson, 2011). The product that is collected after extraction still has many impurities. Sublimation is one way to purify the sample, because caffeine has the ability to pass directly from the solid to vapor and reverse to form a solid all without undergoing the liquid phase. Caffeine has the ability to undergo sublimation under different conditions than the impurities, and can thus be isolated (Tello, 2011). A series of techniques were used to extract pure caffeine from tea leaves. The percent error and percent recovery were calculated to assess how much pure caffeine was obtained, and to account for errors that may have occurred that led to a loss of product.

Materials and Methods:

In order to determine the amount of caffeine in tea, a series of techniques were performed. First, a 50mL beaker was obtained and 2 grams of sodium carbonate were added in order to ensure that caffeine remains present as the free base. The solution was brought to a boil and one bag of Lipton tea was added. 10mL of distilled water was added and the bag was left so steep for 5 minutes. After the 5 minutes had passed, the tea bag was squeezed in order to remove excess water and second bag was added in its place. The second bag was left to steep for 5 minutes and then removed from the beaker after being carefully squeezed to remove excess water. This solid/liquid extractions was performed in order to separated caffeine into the solvent and isolate it from the natural product (Williamson, 2011).

The extract was then poured into a centrifuge tube, or separatory funnel, to ensure proper mixing and placed in an ice bath to cool. 3 portions of 2mL CH₂Cl₂ were then used to extract the caffeine from the solution. The tube was shaken gently after each portion was added in order to mix the solvents while avoiding emulsions. One portion was added, and a transfer pipet was used to remove the bottom layer that contained CH₂Cl₂ and caffeine and place it in a 50mL beaker. This process was repeated two more times for the two remaining portions of CH₂Cl₂ (dichloromethane). There were only a few brown specks in the solution, so a quick swirling motion of the beaker caused them to stick to the sides. The dichloromethane organic layer that contained the caffeine was transferred to another 50mL beaker and a drying agent in the form of calcium chloride (CaCl₂) beads were added in order to remove excess water. Calcium chloride was the drying agent of choice because it is ideal for microscale experiments. After the excess water had evaporated, the caffeine was extracted and placed in a weighed filter flask. Evaporation of the solvent, dichloromethane, would leave a crude version of caffeine. Sublimation would produce a more pure version of caffeine because it would go straight from the solid to vapor form, and then recrystallize again after bypassing the liquid form once more. The caffeine can also be extracted by using a vacuum filtration apparatus in which the organic solution is placed in a vacuum filter, and the caffeine is collected on the filter paper and let to dry (Williamson, 2011).

Results:

Table 1: Measured Weights of Flask, Solution, Flask/Caffeine, and Caffeine Product

Weight of flask, g	29.5780 g
Weight of dichloromethyl/caffeine solution, g	4.960 g
Weight of flask and caffeine product, g	29.643 g
Weight of caffeine product, g	.065 g
Theoretical mass of Caffeine (1 bag)	.055 g (2 bags used)

The various weights of the flasks and solutions were obtained in order to calculate the final amount of caffeine product in grams. The final amount of caffeine extracted could then be used to calculate percent error and percent recovery.

Example

$$\text{Percent Error} = (\text{expected-actual} / \text{expected}) \times 100\% \quad (\text{Eq. 1})$$

$$\text{Percent Error} = (.12-.063/.12) \times 100\%$$

$$\text{Percent Error} = 47.5\%$$

Example

$$\% \text{ Recovery} = (\text{actual}/\text{expected}) \times 100$$

$$\% \text{ Recovery} = .063 \text{ g}/.12 \text{ g} \times 100$$

$$\% \text{ Recovery} = 52.5 \%$$

Table 2: Error and Percent Recovery

% Recovery	59.1 %
% Error	40.9%

The percent yield and percent recovery were calculated by using the final amount of caffeine obtained (.065 g) and by using the known value of caffeine in two tea bags (.11g total, .055 g per bag). The percent recovery makes it possible to understand how much pure product was recovered from the crude product. The percent error accounts for the mistakes that led to a loss of product.

Discussion: The structure of the caffeine extracted from the tea leaves deeply impacts the functions it performs (Wang, 2011) . Essentially, caffeine is a purine with three functional groups: an amine, amide, and an alkene. The basic property of caffeine comes from the lone pair of electrons found around the nitrogen(s) (NCBI, 2013). It is an achiral molecule and does not have any stereoisomers. Caffeine is also a polar molecule; this is evident because of the London dispersion forces, dipole dipole interactions, and hydrogen bonding present when it is in water. It also has a very hydrophobic region (NCBI, 2013). The nitrogen present in caffeine controls solubility. Caffeine is soluble in water at approximately 2.2 mg/ml at 25°C, 180 mg/ml at 80° C, and 670 mg/ml at 100°C (Williamson, 2011). It is an organic molecule that has the properties of an organic amine base (Tello, 2011) .When extracting caffeine, the water was kept at a high temperature in order to increase solubility of caffeine in water to about 670 mg/ml at 100°C. Boiling chips were added to the solution in order to prevent “bumping” and enable the smooth formation of bubbles when boiling occurs. The solution was later cooled to a lower temperature in order to impact the solubility once more and to minimize the attraction to the aqueous layer

while in the separatory funnel (Williamson, 2011). The solution was also cooled before the dichloromethane was added because dichloromethane has a boiling point of 40°C. If the cold water was not added to lower the temperature, the dichloromethane would have evaporated and caffeine would not be properly extracted (Dullo, 2008). During the solid/liquid extraction the solid insoluble material such as cellulose is separated from caffeine and tannins, which are water soluble. In order to isolate caffeine a difference in solubility must occur to separate the tannins into the aqueous layer. Sodium carbonate is added to the extraction medium to ensure that the acidic components in the tea leaves remain water soluble and that caffeine is the free base. Sodium carbonate is basic. Tannins are acidic compounds with a high molecular weight that have an -OH directly bound to an aromatic ring. Because tannins are acidic and can be converted to phenolic salts by deprotonation of the -OH group when a base is added, it is possible to separate the tannins from caffeine (Dullo, 2008). Sodium carbonate serves two main functions: to place caffeine in a more basic environment so that it has a higher affinity for dichloromethane and to cause the tannins to form phenolic salts in the aqueous solution. Adding something basic to caffeine will make it more neutral, and the “like dissolves like” idea can be applied. In this situation, the sodium carbonate acts as a nucleophile and the tannin is an electrophile. Nucleophile attacks electrophile. It is basically an acid/base reaction. The aqueous layer (density of 1 g/ml) contained dissolved tannin salts and chlorophyll. Dipole dipole interactions, London dispersion forces, hydrogen bonding, and ionic bonding with the salts took place.

When dichloromethane was added to extract caffeine from the aqueous solution, two immiscible layers formed: an organic and aqueous layer. In this instance, caffeine is usually a polar substance, but it becomes significantly less polar when it is in a basic solution. Therefore, it is soluble in dichloromethane and suspends in the organic layer. Dichloromethane is an alkyl halide and is denser than water, so it is located at the bottom of the separatory funnel. It has a density of 1.325 g/ml. It had chloro functional groups that make it susceptible to both substitution and elimination reactions (Oneota, 2003). The concentration of the solutes in the organic layer also contributes to the fact that it is located below the aqueous layer. There is a high concentration of caffeine, reactants (because the reaction does not go to 100% completion), and

small amounts of water. The intermolecular forces in the organic layer are van der Waals interactions, dipole-dipole moments, and London forces. Caffeine was extracted with dichloromethane in order to “wash” it three separate times to obtain as much of the pure sample. Emulsions are small droplets of the organic layer that are suspended in the aqueous that are a result of vigorous shaking of the separatory funnel (Williamson, 2011). There are numerous ways to remove emulsions, though the best form is prevention. However, they can break after a sufficient amount of time. The aqueous layer can also be made more ionic, and centrifugation works very well especially on a microscale level.

A drying agent was added to the organic layer because dichloromethane dissolved not only the caffeine, but water as well. The drying agent, anhydrous CaCl_2 was added to remove excess water so that a pure sample of caffeine could be obtained after the solvent evaporated at room temperature (Williamson, 2011). Anhydrous calcium chloride has a high affinity for water, and then reverses back to the hydrous form after it has absorbed the water. Calcium chloride is a preferred drying agent because the pellets form clumps when excess water is present that make it simple to identify how much drying agent is needed (Williamson, 2011). The pellets stopped clumping together when excess water was removed. It is also very rapid, effective, and ideal for microscale experiments. In order to remove the dichloromethane, the beaker could be placed in a hot water bath so that the solvent would evaporate and leave a pure sample. Sublimation is a technique that may have been used to produce a purer caffeine sample, but it could have led to a higher loss of product. Liquid-liquid extractions were used to transfer a solute from one solvent to another and isolate desired product.

A total of 110 mg of caffeine could have possibly been extracted from the two bags of tea. The weight of the caffeine extract was .065 g. The calculated percent recovery was 59.1%. This was the amount of caffeine extracted from the crude caffeine in the tea bags. This demonstrates that there was a significant amount of product lost throughout the procedure. It is also important to consider that the reaction cannot go to completion, so 100% yield is not possible. A loss of product could have occurred due to emulsions and due to not thoroughly “washing” with dichloromethane to extract as much caffeine as possible. There was a lot of transfer throughout the procedure, which presented many opportunities to lose product. It is

also possible that the concentration of caffeine was not high enough because too much water was added. A systematic error with the scales was observed due to a lack of calibration, this could have affected the measurement of the final product. Another source of error could be the theoretical amount of caffeine in the tea bags, if it was more or less due to random error, the percent recovery would be calculated differently. The overall percent error was about 40.9 %. This number could be skewed due to measurement errors of the crude product. In order to reduce sources of error in the future, two trials could be done. It is also possible to use a different source of caffeine, and to ensure all techniques are performed properly.

Conclusion: Overall, a total of .065 g was obtained from a possible amount of .055 g per tea bag, or .11 g total. The total percent recovery was 59.1 %. This number reflects on how accurate the procedure was performed. It was not possible to obtain 100% recovery because the reaction never goes 100 % to completion and because of material loss through transfer during the procedure.

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