Using Essential Laboratory Techniques to Separate, Purify, and Identify Various Substances

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Overview:

During a chemical reaction, the bonds between the atoms in the reactants are broken, and the atoms bond in new ways to form products that are physically and chemically different from the reactants. If you wish to *separate, purify,* and *identify* your desired product, how do you accomplish this so that it is not mixed with leftover reactants, the solvent, the catalyst, or other products that you may not want? Here we report a series of investigations that utilize several laboratory techniques, including thin layer chromatography (TLC), melting point determination, liquid-liquid extraction, recrystallization, fractional distillation, and gas chromatography (GC), that were used to separate, purify, and identify products of several different chemical reactions. Learning different techniques are important laboratory skills that can be carried out on in an industrial setting. Of course all of these techniques can be employed on small scale in a laboratory as demonstrated in this lab report, but they are essential to large-scale industrial processes since many chemical products are made by a combination of these aforementioned processes. This all relates back to the driving question of our course which is "How do you take a simple chemical reaction and turn it into an efficient industrial process?" because it is *these* essential laboratory techniques that when applied can lead to simple chemical reaction being turned into an efficient large industrial process

Part 1 - Identifying An Unknown Analgesic

Background:

Thin layer chromatography (TLC) is an analytical technique used to separate mixtures of organic compounds according to a compound's affinity for the mobile and stationary phase, which affect the speed at which it migrates up the plate. TLC can also be used to identify compounds by comparison with known samples, to check the purity of a compound, and to track the progress of a reaction to see if there were any reactant(s) that remained. The technique is a standard analytical tool used in industrial processes because it is fast, easy, and inexpensive, and it proves to be extremely useful in the chemical pharmaceutical industry as it can determine production and quality assurance of substances as well as the purity of a drug sample. In thin layer chromatography, there are two different phases as mentioned above: the stationary phase and the mobile phase. The stationary phase is a thin layer of the adsorbent, which is typically silica or alumina, coated on a glass sheet, and the mobile phase is the liquid solvent, commonly referred to the eluent.

Compounds are loaded onto the silica gel coated TLC plates, and they are placed into a development chamber where they encounter the mobile phase (solvent) and slowly rise due to capillary attraction. These compounds travel a specific distance that is relative to the solvent front, and more than one compound can be separated on a TLC plate once the mobile phase is preferred for every compound. In general, different compounds move at different rates because of their attractions for the mobile and stationary phases. For example, if one compound was more polar than another, it would be more attracted to the very polar silica gel (stationary phase) and move less up the plate than the other compound due to its stronger attractions to the plate. Also,

if the solvent (mobile phase) was less polar, the more polar compound would not be as soluble and move less far with the solvent. Overall, the goal of TLC is to obtain well-defined, well-separated spots if your sample is pure, but if a sample is a mixture of compounds it will separate into a series of spots at varying distances up the plate. A UV light source is used to visualize these spots on the TLC plates as the chemical deposits will appear as dark spots against a bright background.

Results from thin layer chromatography are expressed in terms of the retention factor (R_f value), which is a ratio calculated by dividing the relative distance that the sample traveled by the distance that the solvent travelled at the end of the experiment. The R_f values allow the experimenter to compare the different compounds given that the eluent remains constant, which can allow them to identify these compounds as well. When comparing two different compounds that were tested using the same eluent, the compound with the larger R_f value is less polar because it does not adhere to the stationary phase as long much as the more polar compound, which would have the lower R_f value since it does.

Just as thin layer chromatography can be used to identify a substance and test the purity of a sample, so can melting point temperature. The melting point of a substance is the temperature range over which it begins to melt and when it has completely melted, and it is an intensive property. When a solid is heated, the heat energy that is added to the system is transformed to kinetic energy, which causes an increase in particle motion within the sample. The increased particle motion is what allows for the particles to partially overcome the attractive forces that keep them in place as a solid and begin to melt. A substance has a sharp melting point if its melting temperature range is less than 2ºC, and if the range is greater than 2ºC then the

substance has a broad melting point. That being said, pure compounds typically have a sharp melting range because all of the particles are the same and the attractive forces are maximized, however, most impure or mixed substances have a broad melting point range because there are weakened attractions since the structure is no longer regular causing the melting point of the substance to decrease. Also, since different parts of the sample may contain different amounts of impurities, the amount of energy required to melt it will vary and the range of temperatures will be greater. It is important to note that simply ascertaining the melting point range of an unknown substance is insufficient to identify the unknown because other substances can have the same, or very similar melting points, which is why melting point temperature should be used in conjunction with another lab technique.

Data Analysis:

In this experiment, we used both the technique, thin layer chromatography, and melting point temperature to identify the active ingredients in an unknown over-the-counter medicine tablet by collecting data on known medicines and then comparing their data to that of the unknown. The tablet which we tested contained one or more of the following active ingredients found in various over the counter tablets, including acetylsalicylic acid (found in aspirin), acetaminophen (found in Tylenol® and Excedrin®), caffeine (found in Excedrin®), and/or ibuprofen (found in Advil®). As a group, we followed the procedure and grinded a tablet of each known sample into fine powder and dissolved each of them, along with our unknown sample, in ethyl acetate. We loaded each sample onto its respective mark on each of the three plates that we prepared and placed each plate in a separate chamber that contained a different solvents. We tested three different developing solvents, ethyl acetate, hexane, and 1:1 ethyl acetate/hexane, because it would allow us to determine which solvent produced the best separation. Then, we

could use the better TLC plate to calculate the R_f values for each spot and use that data as one part of identifying the active ingredient(s) in the unknown tablet.

Once we completed our TLC technique and let all three of the plates dry, we observed them all under a UV lamp and lightly outlined the spots with a pencil. We collected the distance that each sample travelled from the origin and the solvent front for each of the TLC plates, so that we could determine which solvent would help us identify the unknown sample. We found that hexane did not display separation in any of the active ingredients or the unknown sample as the spots remained at the origin of the TLC Plate as shown in Figure 1. The 1:1 mixture of ethyl acetate/hexane did display separation between all of the spots, but the distances that the samples travelled were all rather close to one another as shown in Figure 1. We believed that the 1:1 mixture of ethyl acetate/hexane was not a preferred mobile phase for these five samples since the spots were not *well-defined* nor *well-separated*. The ethyl acetate TLC plate, on the other hand, exhibited the best separation of the four active ingredients as well as the unknown sample as the spots were not on the origin like what occured to the samples on the hexane TLC plate, and they were more separated than the spots on the 1:1 mixture on the ethyl acetate/hexane TLC plate because there was greater differentiation in the distances that the four active ingredients and the unknown travelled (shown in Figure 1). This would make the comparison of the R_f values of the samples much easier as there was a *better* range than the R_f values of the samples taken from the 1:1 mixture of the ethyl acetate/hexane TLC plate.

This is why as a group we decided that ethyl acetate was the preferred mobile phase for these samples as the spots were *well-defined* and *well-separated,* so we calculated the R_f values for each spot on the ethyl acetate TLC plate. We calculated that the R_f value of the unknown

sample was 0.425, and it was closest to the R_f value of acetaminophen, which was 0.401. The R_f values of the other three active ingredients were not relatively close to the R_f value of the unknown as shown in Figure 2. Besides using thin layer chromatography as a technique to identify the unknown sample, we also determined the melting point temperature of each of the five substances, so that we would have more evidence to conclude what active ingredients were in the unknown sample. As seen in Figure 3, from using the Melting Station, we were able to determine the melting point range for each of the active ingredients and the unknown, so we could compare the melting temperature of the unknown to the melting point temperature of the other four known active ingredients. We conducted two trials on the melting point temperature of the unknown, so that the *hopefully* the second trial would be more accurate; for trial 1 the melting point temperature was 169.1ºC - 174.5ºC, and for the second trial the melting point temperature was 169.7ºC - 174.5ºC, which had a slightly smaller range. Based on our data shown in Figure 3, we observed that the melting point temperature of the unknown was very similar to the melting point of acetaminophen, which was 170.0ºC - 174.0ºC. It is important to note that the accepted melting point temperature of acetaminophen is 168ºC -172ºC, so even our melting point temperature for that drug was not *that* off as the range was still 4ºC.

Both our data collected from thin layer chromatography as well as melting point temperature indicated that the unknown sample contained acetaminophen as their R_f values from TLC were *relatively* close to one another as displayed in Figure 2, and their melting point temperatures were almost identical as seen in Figure 3. Our data collected from TLC and melting point temperature gave us reason to conclude that it was acetaminophen as the R_f value of the

unknown was not similar to the R_f value of the other three active ingredients and neither was its melting point temperature.

The Observations of Four Active Ingredients and the Unknown from Using TLC with Three Different Solvents

Substances	***Distances Traveled on Hexane TLC Plate (cm)***	***Distances Traveled on Ethyl Acetate TLC Plate (cm)***	***Distances Traveled on Hexane/Ethyl Acetate TLC Plate (cm)***
Solvent Front (cm)	7.70 cm	8.00 cm	7.81 cm
1 (Acetaminophen)	0.00 cm	3.21 cm	0.70 cm
2 (Acetylsalicylic acid)	0.00 cm	5.23 cm	2.58 cm
3 (Caffeine)	0.00 cm	1.29 cm	0.30 cm
4 (Ibuprofen)	0.00 cm	6.01 cm	3.80 cm
5 (Unknown)	0.00 cm	3.40 cm	0.61 cm

Figure 1: This is our data collected from each of the TLC plates, and it displays the solvent front in centimeters as well as the distances that each of substances travelled with the three different mobile phases (hexane, ethyl acetate, and 1:1 hexane and ethyl acetate) in centimeters.

The R_f Values of the Four Different Active Ingredients and the Unknown from the Ethyl Acetate TLC Plate

Substances	**Distance Traveled by Solute (cm)/ Distance Traveled by Solvent (cm)**	**R_f Values**
1 (Acetaminophen)	3.21 cm/8.00 cm	0.401
2 (Acetylsalicylic acid)	5.23 cm/8.00 cm	0.654
3 (Caffeine)	1.29 cm/8.00 cm	0.161
4 (Ibuprofen)	6.01 cm/8.00 cm	0.751
5 (Unknown)	3.40 cm/8.00 cm	0.425

Figure 2: These are the calculated R_f values for each of the substances when ethyl acetate was the solvent used. The R_f values were calculated by dividing the distance that the solute traveled in centimeters by the distance traveled by the solvent in centimeters.

The Melting Point Temperature Ranges of the Four Different Active Ingredients and the Unknown Taken from Melting Station

Substances	Melting Point Temperature Ranges (ºC-ºC)
1 (Acetaminophen)	170.0 ºC - 174.0 ºC
2 (Acetylsalicylic acid)	134.5 ºC - 140.3 ºC
3 (Caffeine)	217.3 ºC - 244.0 ºC
4 (Ibuprofen)	77.6 ºC - 81.5 ºC
5 (Unknown)	Trial 1: 169.1 ºC - 174.5 ºC Trial 2: 169.7 ºC - 174.5 ºC

Figure 3: These are the melting point temperature ranges for each of the substances that was determined by using the Melting Station. Two trials were conducted on the unknown sample (5) with the hopes that the second trial would be more precise than the first.

Conclusion:

By using thin layer chromatography and melting point temperature in conjunction, we were able to conclude that the unknown sample contained acetaminophen as their R_f values and melting point temperature ranges were similar to one another. Thin layer chromatography allowed us to compare our unknown sample to other known samples with the R_f values of the spots on the plate that used ethyl acetate, which was the better solvent out of the three as it produced the best separation. We could have merely identified the active ingredient in the unknown sample from just that, however, by employing melting point temperature as well, we were able to more conclusively support our claim that the unknown sample did, in fact, contain acetaminophen due to their similar melting point ranges as well. In general, we saw how effective both of these techniques can be when used during an experiment as they helped us identify an unknown substance, but there are some shortcomings to these techniques. For example, thin layer chromatography is imprecise as you do estimate the relative distance that each sample travels up the plate, and in order to identify an unknown, you need *known* samples

for comparison, and for melting point temperatures, some substances may have similar melting point ranges, so it may not be the best technique to use to identify an substance. Yet, chemical processes have shown us that thin layer chromatography is still useful as it fast, inexpensive, and can be used to check the progress of chemical reactions.

Part 2 - Recrystallization of Benzoic Acid

Background:

Recrystallization is a technique for purifying solids that is based upon the differences in solubility between your desired product and impurities within a sample. The technique of purification is based on the principle that the solubility of a solid in a given solvent increases as temperature increases and decreases as the solution cools. It is important to choose a proper solvent because the desired substance should not be soluble at room temperature and soluble at higher temperatures. Rather, the desired substance should be insoluble at room temperature and soluble at high temperatures, so it can be purified. Ideally, it is your impurity in your sample that you want to be soluble at all temperatures or insoluble at all temperatures.

When you dissolve an impure sample, you want to utilize the minimum amount of hot solvent that will completely dissolve the sample to be purified, so that when the solution begins to cool down the desired substance will recrystallize before the impurities in the sample and incorporate only the particles that have an affinity for the particles that make up the crystal lattice. The rate of cooling is a key factor that affects the purity of the crystals as a slow cooling process allows for nearly pure crystals of the compound to form, and a fast cooling process causes the impurities to precipitate out of the solution along with the desired product. Given that recrystallization is carried out properly, the impurities that do not fit into the crystal lattice will remain in the solution, and then a filtration process must take place to separate the crystals at this

point, such as vacuum filtration. The filtration process can be repeated to ensure that *all* of the crystal are removed from suspension. Recrystallization can be used in industrial chemical processes to purify products as it is a very effective in obtaining a pure sample of some product, and in large-scale reactions it is important that all samples are pure because impurities can compromise the sample and be unsafe.

Data Analysis:

In part 2 of the series of investigations, we used recrystallization to purify an impure sample of benzoic acid and tested the purity of benzoic acid (our recrystallized product) by analyzing its melting temperature and comparing it to the accepted melting temperature of pure benzoic acid. Before we began the process of purifying the contaminated benzoic acid sample, we had to determine which solvent worked best to obtain the benzoic acid from that contaminated sample. To do this, we tested the solubility of pure benzoic acid in three different solvents, 2-propanol, acetone, and water, at room temperature, in a hot water bath, and in an ice water bath. The parameters of the proper solvent was that the pure benzoic acid would be insoluble at room temperature and soluble in the hot water bath and ice water bath, so that we would *essentially* be able to dissolve our contaminated benzoic acid sample when heated and then allow the (hopefully) pure benzoic acid crystals to form overnight since the pure benzoic acid is insoluble at room temperature.

From our testing on choosing a recrystallization solvent, we determined that the best solvent to use for our contaminated benzoic acid sample was water because it met the parameters mentioned above. It was insoluble at room temperature and soluble in an hot water bath and ice bath while the acetone and 2-propanol were soluble at room temperature, in an hot water bath, and in an ice water bath as seen in Figure 4. Thus, we dissolved 0.760 gram of the contaminated

benzoic acid sample in a minimal amount of water and gently heated it until it was dissolved. We let the solution slowly cool to room temperature by leaving it overnight in the fume hood to form crystals and then collected our crystals using vacuum filtration, which is a technique for separating a solid product from a liquid as the solid gets trapped in the filter paper while the liquid is drawn through a funnel into a flask by a vacuum. This process was repeated several times to ensure maximum purity of the benzoic acid crystals. Afterwards, we put the solid into the drying oven, but because we left it in the drying oven for too long our benzoic acid vaporized. Because of that we had to repeat this process again, but this time with 0.807 gram of the contaminated benzoic acid sample.

The second time around, we obtained our benzoic acid crystals and put them in the drying oven to ensure that the leftover water particles evaporated, and the weight of our recovered solid was 0.500 g. That meant using our 0.807 gram of the contaminated benzoic acid, we recovered 0.500 gram of benzoic acid, and we calculated our percent recovery of benzoic acid by dividing the amount of the solid we collected by the amount of the sample that we started with and multiplied that value by 100% and determined that our percent recovery of the pure benzoic acid was 62.0%. We tested its melting point temperature of our recovered benzoic acid to conclude whether it was *actually* pure by comparing its melting point temperature range to the accepted melting point of pure benzoic acid. The accepted melting point of pure benzoic acid is 122.4ºC, and our experimental melting point of the recrystallized benzoic acid was 123.6ºC - 128.4ºC. The range for our experimental melting point was 4.8ºC, which is greater than 2ºC, so we determined that our recrystallized benzoic acid was slightly contaminated as it had a relatively large melting point temperature range, which indicated that it was still slightly impure because other

substances caused it to have a broad melting point range by weakening the attractions of the irregular structure.

The Observations of the Solubility of Pure Benzoic Acid in Three Different Solvents At Room Temperature, in an Hot Water Bath, and in an Ice Water Bath

	Room Temperature	**Hot Water Bath**	**Ice Water Bath**
Water	insoluble	soluble	soluble
Acetone	soluble	soluble	soluble
2-propanol	soluble	soluble	soluble

Figure 4: This shows the solubility of the pure benzoic acid in three different solvents (water, acetone, 2-propanol) at room temperature, in an hot water bath, and in an ice water bath.

Conclusion:

By determining the proper solvent to recrystallize our sample of contaminated benzoic and carrying out the process of recrystallization, we were able to compare the experimental melting point of our recrystallized product of benzoic acid to the accepted melting point of pure benzoic acid to determine its relative purity. Recrystallization enabled us to purify the contaminated benzoic acid sample based on the differences in solubility between our desired product (benzoic acid) and the impurities once we determined that water was the better solvent because the pure benzoic acid would be insoluble at room temperature and soluble in the hot water bath and in an ice water bath. We were able to discern the purity of our recrystallized product by comparing its melting point range to the melting point of benzoic acid and conclude its impurity based on how similar it was to the accepted melting point and whether it had a broad melting range. We concluded that our recrystallized benzoic acid was slightly impure due to its relatively large melting point temperature range that was greater than the accepted melting point temperature of pure benzoic acid. As a group, we observed how effective recrystallization can be when carried out correctly as you can obtain a pure sample of some product on a small

laboratory scale, but it is just as effective in large-scale industrial processes as well. However, there are some disadvantages of recrystallization as it takes a long time to occur because you first have to determine which solvent should be used and that is a matter of trial and error of testing various solvents. A key component of large-scale industrial processes is being timely, so recrystallization may not be preferred, however, this technique is still successful at purifying impure samples.

Part 3 - Separation of Organic Compounds By Liquid-Liquid Extraction

Background:

Liquid-liquid extraction is another separation technique that is used to separate components in a mixture based on their differences in solubilities in two or more *immiscible* solvents as well as the solvents' densities. The reason why the solvents must be immiscible is because you want to form separate layers, so that they do not mix with one another to produce a homogeneous solution as you will not be able to separate the components in a mixture. Rather, you want to pick solvents that have different polarities because it is that property that determines whether they are immiscible or miscible. For example, if you had two solvents that were both very polar then they would be attracted to one another and not form separate layers, so there would be no way of separating the components in a mixture. The technique works well if your target components of a mixture are soluble in different solvent layers; for example, if your reaction mixture contained two substances then you would want them to partition into different layers where they were more soluble, so that they can be separated. The most dense solvent would be at the bottom while the least dense solvent would be at the top. In general for this technique, at least in a small-scale laboratory, you use a separatory funnel and add a solvent that one of the substance is more soluble in than the other substance and shake the solution

containing the reaction mixture with an immiscible solvent in which one substance is *more* soluble than it is in the starting solution. Upon standing, the solvents form two layers that each contain a different desired substance that can be separated. If one wanted to ensure that the two substances were completely separated, they could repeat this process several times to ensure accuracy.

This technique of liquid-liquid extraction can be used to separate organic acids from neutral compounds by utilizing aqueous solutions of different pH values. The method is based on the fact that most organic compounds are more soluble in organic solvents than they are in water. Organic carboxylic acids are highly soluble in dilute aqueous sodium hydroxide because the acid becomes sodium carboxylate salt, which is the conjugate base of that acid. Therefore during liquid-liquid extraction, the carboxylic acid can be selectively separated from its reaction mixture by dissolving the mixture in an organic solvent that is immiscible with water and then adding an aqueous solution of sodium hydroxide. When the carboxylic acid becomes sodium carboxylate salt, it will dissolve in the basic aqueous layer while the other components of the mixture will remain dissolved in the organic solvent. When the desired layer that contains the sodium carboxylate salt is drained off, it can be acidified so that the sodium carboxylate salt can be converted back to the carboxylic acid, which as mentioned earlier is not soluble in water. Therefore, the acid will precipitate from the solution and be separated from the mixture.

Liquid-liquid extraction is a standard separation technique used in industrial processes as it is can separate components in a mixture based on their differences in solubilities and is cost effective. It works on the principle of chemical structure and attraction differences, which is why liquid-liquid extraction is so suitable for many chemical industrial processes. There is another

technique for separation, which is distillation, and it is frequently used in industrial processes over liquid-liquid extraction.

Data Analysis:

During the experiment pertaining to liquid-liquid extraction, we separated the components of a mixture containing benzoic acid and naphthalene (both organic compounds) and analyzed them both by determining their melting point temperature and percent recovery. We first dissolved our sample mixture of benzoic acid and naphthalene in ethyl acetate and then we placed it into the separatory funnel, which we poured sodium hydroxide (NaOH) into. The reason why we added aqueous sodium hydroxide, and not just water, to the mixture dissolved in the ethyl acetate is because benzoic acid, which is evidently an acid, is insoluble in water because it is less polar than water. It is only soluble in water at high temperatures because the attractions between water molecules weaken and attract the less polar benzoic acid. However, at room temperature when we added the base, sodium hydroxide, to the mixture that contained benzoic acid, the base reacted with the benzoic acid and produced sodium benzoate, which is soluble in water. This process was a acid base neutralization reaction, and the chemical equation is referred to on the flow chart on lab page 65. This meant that the benzoic acid was in the aqueous layer, and the other compound, naphthalene, was in the other layer since it had no attractions to the aqueous layer and would not react with the sodium hydroxide.

After that we followed the rest of the procedure that instructed us how to drain the two layers and acidify the aqueous layer that contained the benzoic acid as seen in the flow chart on lab page 65. The aqueous layer with benzoic acid was drained first as it was more dense, and the organic layer was drained afterwards as it was less dense. We added hydrochloric acid to the aqueous layer so that it would react with the sodium benzoate to produce the original form of

benzoic acid as well as the by products, which were sodium chloride and water (refer to lab page of 65 to see chemical equation). We collected the solid using vacuum filtration several times as our crystals were small and would escape through the filter and saved it for melting point analysis, which was conducted later. We drained the organic layer that contained naphthalene into a flask with anhydrous sodium sulfate to get rid of any remaining sodium benzoate and gravity filtered the flask to separate sodium sulfate. The last step of this experiment was evaporating the ethyl acetate, so that we would be left with naphthalene.

The mass of our recovered benzoic acid was 0.050 gram, and the recovered mass of our recovered naphthalene was 0.505 gram. We calculated the percent recovery of each substance by dividing the mass of the solid we collected by the half of the mass of the amount of the sample that we started since the sample mixture was half benzoic acid and half naphthalene and multiplied that value by 100%. Our percent recovery of the benzoic acid was 9.9%, and our percent recovery of our naphthalene was 98.2%. We deduced that our percent recovery of our benzoic acid was so small because we may have lost crystals during the vacuum filtration process as they were so small that they fell through the filter paper into flask *many* times. This was not the case for naphthalene as the crystals were large once the ethyl acetate evaporated.

Next, we tested the melting point temperature of both benzoic acid and naphthalene and compared them to those pure compounds' accepted melting point temperatures. The accepted melting point of pure benzoic acid is 122.4 ºC, and our experimental melting point of the recrystallized benzoic acid was 123.4 ºC - 126.5 ºC. The range for our our experimental melting point was 3.1 ºC, which is slightly greater than 2 ºC, so we determined that our recrystallized benzoic acid was possibly a little impure. However, our experimental melting point was quite

similar to the accepted melting point, which allowed us to conclude that our sample was *actually* benzoic acid. Lastly, the accepted melting point of pure naphthalene is 80.26 ºC, and our experimental melting point of the recrystallized naphthalene was 77.5 ºC - 83.6 ºC. The range for our our experimental melting point was 6.1 ºC, which is greater than a 2 ºC range, so we determined that our recrystallized naphthalene must have been slightly impure as well. Yet, we can still determine that our separated naphthalene *most likely* naphthalene as our experimental melting point was close to the accepted melting point of naphthalene.

Conclusion:

By using liquid-liquid extraction, we were able to separate benzoic acid and naphthalene from a mixture and confirmed that it was each compound by using melting point temperature data. Liquid-liquid extraction allowed us to complete this process based on the differences in solubility of the organic compounds and the densities of the solvents, benzoic acid and naphthalene, in two immiscible layers. Also, by determining the melting point range of each sample, we were able to determine how pure our sample was and more *conclusively* determine whether the substance was correct by comparing our experimental melting points to the accepted melting points of the pure compounds. We found that we did not separate much benzoic acid, and our sample must have been slightly impure due to its range in melting point temperature; similarly, we did separate a large quantity of naphthalene, but its range was large meaning that our sample was most likely impure. In general, my group understood how effective liquid-liquid extraction is because you it allows you to separate mixtures based on the components' solubilities in the *different* layers, and this technique can be applied to large-scale industrial processes as well. Some drawbacks of this technique is that sometimes during the process of

filtering your compound, the crystals can be too small and then wasted, so if this were to happen in industrial processes, the plants would be wasting product, thus, wasting money.

Part 4 - Distillation of Esters and Analysis By Gas Chromatography

Background:

Distillation is a technique used to separate mixtures based on the differences in the conditions required to change the phase of the components in the mixture and can be applied to mixtures of liquids and solids, mixtures of two or more liquids, and mixtures of two or more gases. Two liquids in one mixture can be separated through distillation once the two liquids have differing boiling points (based on intermolecular attraction) with a difference that is between 20°C to 25°C in order for this technique to work effectively because if not both liquids will boil and condense together. When the mixture being distilled is heated, the liquid that has the lowest temperature will begin to boil and form vapors that travel down the condenser and cool down until it is a liquid. As this process progresses, the concentration of the liquid with the lowest boiling point will steadily decrease as the temperature increases. The temperature shown on the thermometer within the apparatus plateaus and then decreases in temperature because all of the gas of the liquid with lower boiling point temperature has condensed. Eventually, the temperature will increase again until it reaches the boiling point of the next liquid in which that liquid boils, forms vapors, and then condenses into a liquid. If one conducted fractional distillation, they would follow the steps mentioned above but also collect the distillates into a number of different parts. It is important to know that the distillate is richer in the more volatile component.

Distillation is a core process that is fundamental to any process plant as it is used to separate components based on their boiling points, which is why it is so *frequently* used. One

industrial application of distillation is refining crude oil through fractional distillation. Crude oil contains different components with unique sizes, boiling temperatures, and condensing temperatures, so they are separated based on their boiling temperatures and collected in fractions. The components with lower boiling points take less time to turn into gas than the components with higher boiling points since boiling point is based on intermolecular attractions, and the components with the lower boiling points require less energy to break these attraction than the components with the higher boiling points do. The collected liquid fraction may pass through condensers that cool them further, and then they can be stored or processed some more.

Just as distillation can be used to separate components in a mixture based on their boiling points, gas chromatography can also be used to separate and analyze the components of mixtures that can be vaporized without decomposition. Similar to thin layer chromatography (TLC), gas chromatography involves compounds carried through a stationary phase by a mobile phase that is pressurized and pushes the mobile phase through the column. During gas chromatography, mixtures are vaporized and travel through a coated column (stationary phase that is the equivalent to the silica gel on TLC plates) carried by an inert non reactive gas (mobile phase). The stationary phase affects how long it takes the substances to emerge because if there is a stronger attraction between the stationary phase and the substances in gas form, they will adhere to the column due to the strength of the intermolecular interactions and take a longer amount of time to emerge. The binding between the substances and the column is determined by the number and strength of the intermolecular attractions between them. Another factor that allows for better separation of substances in gas chromatography is lowering the pressure of the carrier gas and the initial temperature of the column because the time in which the substances reach the

detector would be based upon their melting point as the pressure of the carrier gas would not be great enough to push all of the substances to the detector at once and the temperature would not be great enough to vaporize all of the substances.

The Vernier Mini Gas Chromatograph uses a metal column in which the stationary phase is a nonpolar coating, and the mobile phase is the atmospheric air. When the substance is pushed out of the chromatography column, it is detected by a chemical sensor. The chemical sensor produces electrical responses proportionate to the concentration of the substances, and the program generates a peak for when the substance reaches the detector. The time that takes for a substance to come out of the column and reach the detector is known is the retention time. Substances that has a larger retention time has a higher boiling points. Also, the area under the peak (the integration) indicates the relative percentage of each substance in a sample. Thus, gas chromatography can indicate what substances are present as well as their relative amounts, which is why this process is so helpful in industrial processes. It is a better analytical tool than thin layer chromatography because of its precision and its ability to figure out the relative amount of each substance in a mixture, which thin layer chromatography *cannot* do.

Data Analysis:

In the final part of this series of investigations, we distilled a mixture of ethyl acetate and butyl acetate, collected the distillate in seven fractions, and analyzed these fractions using gas chromatography to determine their retention times and peak integration. The boiling point of ethyl acetate is 77 °C, and the melting point of butyl acetate is 125 °C. Thus, as a group, we determined that the ethyl acetate would be the liquid to boil first since it had the lower boiling point, and it would have the biggest relative amount in the first couple of fractions until it became less concentrated in the mixture. Then, the butyl acetate would have the biggest relative

amount in the last couple of fractions because it was less volatile (refer to lab page 63 for further observations of distillation of ethyl acetate and butyl acetate). After the distillation of the mixture of ethyl acetate and butyl acetate was completed, we did gas chromatography to analyze the effectiveness of distillation and determine whether the ethyl acetate and butyl acetate were properly separated.

Prior to conducting gas chromatography on our fractions, we had conducted it on ethyl acetate, butyl acetate, and a 50:50 mixture of ethyl acetate and butyl acetate to determine their retention times and peak integration to use in comparison to the gas chromatography results of our seven fractions. The retention time for the ethyl acetate was 1.805 minutes, 5.215 minutes for butyl acetate, and 1.810 minutes in the first peak (ethyl acetate) of the 50:50 mixture of ethyl acetate and butyl acetate and 4.640 minutes in the second peak (butyl acetate). The reason why we thought that the retention time of ethyl acetate was less than the retention time of butyl acetate was because the boiling point of ethyl acetate is lower which means that it spends less time at the beginning of the column and reaches the detector faster than the butyl acetate that spends more time at the beginning of the column. As seen in Figure 5, for all of our fractions there was range in retention times for the ethyl acetate and butyl acetate, but the retention times of the butyl acetate in all of the fractions were larger than the retention times of the ethyl acetate due to higher boiling point. Also, the retention time values that we determined from the gas chromatograms of ethyl acetate and butyl acetate before our fractions were very similar to the retention times of ethyl acetate and butyl acetate from our fractions.

When analyzing the peak integration of our fractions (shown in the Figure 5), we saw that in the later fractions, the percent area of ethyl acetate decreased, and the percent area of the butyl

acetate increased. Therefore, the relative amount of ethyl acetate was lesser than the relative amount of butyl acetate in the later fractions because towards the end of the distillation process, the mixture was mostly butyl acetate as the mixture heated up and moved away from the melting point temperature of the ethyl acetate. This meant that most of the ethyl acetate had boiled out and been distilled meaning that the last fractions would be mostly butyl acetate, and this is supported by our last fraction whose percent area is 100% meaning that it was *purely* butyl acetate (shown in Figure 5). Although our fractions containing ethyl acetate and butyl acetate were very separated, to achieve better separation in future experiments, we could lower the temperature of the heat applied to the flask containing the mixture so that there isn't some butyl acetate in the first fraction because of refluxing. Also, we could distill each individual fraction to further separate the ethyl acetate and butyl acetate.

The Retention Times and Percent Area of Each Fraction From Distillation of Mixture of Ethyl Acetate and Butyl Acetate Received From Gas Chromatography

Fraction Number	Retention Time	Percent Area Per Peak
1	Peak 1: 1.800 min Peak 2: 4.140 min	Peak 1: 91.54% Peak 2: 8.46%
2	Peak 1: 1.900 min Peak 2: 4.415 min	Peak 1: 74.50% Peak 2: 25.50%
3	Peak 1: 1.865 min Peak 2: 4.485 min	Peak 1: 55.01% Peak 2: 44.99%
4	Peak 1: 1.895 min Peak 2: 4.740 min	Peak 1: 19.18% Peak 2: 80.82%
5	Peak 1: 1.705 min Peak 2: 4.720 min	Peak 1: 4.79% Peak 2: 95.21%
6	Peak 1: 1.745 min Peak 2: 5.115 min	Peak 1: 0.45% Peak 2: 99.5%

7	Only one peak: 5.315 min	Only one peak: 100%

Figure 5: This is our data collected from gas chromatography about the retention times and percent area per peak for each of the seven fractions from distillation.

Gas Chromatogram of Fraction 4 from Distillation That Shows its Retention Times and Percent Area of Peaks

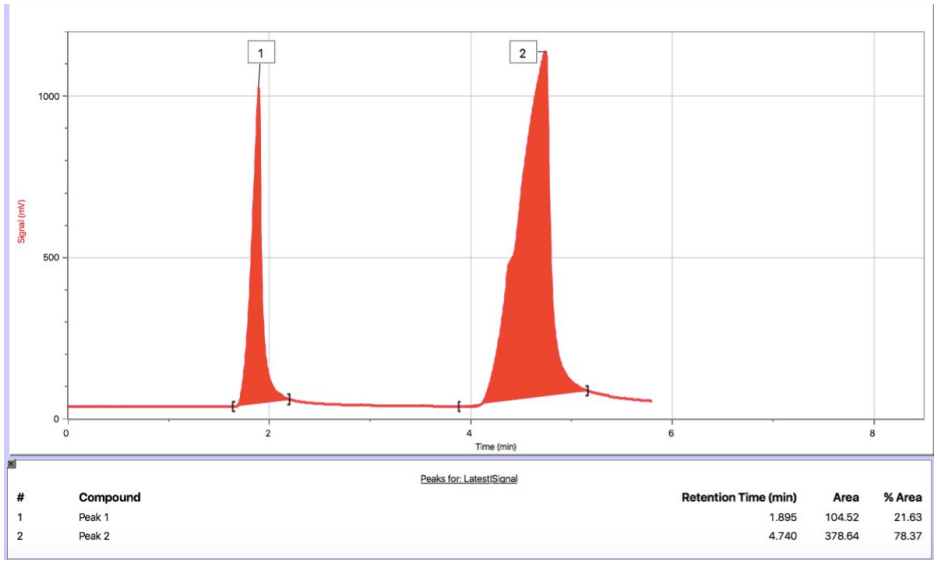

Figure 6: This is an example of our gas chromatograms for each of our fractions that details the retention time and percent area for each of the peaks. As the fraction number increased, the percent area of ethyl acetate decreased while the percent area of butyl acetate increased.

Conclusion:

By using distillation and gas chromatography in conjunction, we were able to separate a mixture of ethyl acetate and butyl acetate into different fractions and analyze how separated these two substances were. The gas chromatograms of the fractions determined the retention times of the ethyl acetate and the butyl acetate, and the peak area of the ethyl acetate and the butyl acetate. Based on the peak area of the ethyl acetate and butyl acetate of each fraction, we were able to conclude that our distillation was relatively effective as the first fraction was 91.54% ethyl acetate meaning that it had been separated properly and was the concentrated as hypothesized, and the last fraction was 100% butyl acetate meaning that it was separated very well as predicted. In general, we saw how effective distillation and gas chromatography are as

techniques during an experiment as they helped us separate a mixture and analyze how separated the ethyl acetate and butyl acetate, in fact, were.

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