Part 2: Column Chromatography and Thin Layer Chromatography (TLC)

Background

Chromatography is one of the most ubiquitous methods of analyzing and purifying organic compounds. This technique, originally used to separate plant pigments, encompasses a variety of sophisticated methodologies that allow for the separation, isolation, and identification of the components of a mixture. While there are many types of chromatography, the fundamental basis for this technique is the distribution of the individual components of a mixture between two phases: the *stationary phase* and the *mobile phase*. For any given compound (**A**), there is equilibrium between the compound on the stationary phase and the compound in the mobile phase. This equilibrium is determined by the polarity of the stationary and mobile phases, and the polarity of the compound itself. In gas chromatography, the equilibrium is determined primarily by temperature.

$$A_{(mobile)} \rightleftharpoons A_{(stationary)}$$

As the name implies, the stationary phase is a non-moving substance (often SiO_2 or Al_2O_3) to which the components of a mixture may adsorb. It can exist in a variety of forms, but is commonly contained in a column or spread in a thin layer over a glass or plastic backing. The mobile phase percolates over the stationary phase and may be either a gas, as in gas chromatography, or a liquid as in column or thin-layer chromatography. A compound dissolved in the mobile phase is carried along in the direction of the flow. A compound adsorbed on the stationary phase does not move.

The individual components of a mixture also exist in equilibrium between stationary and mobile phases. In many cases, the mixture is first loaded on the stationary phase and then the mobile phase is added. As the mobile phase flows over the stationary phase, it carries with it all components of the mixture in the direction of flow. Since each component has a different affinity for the stationary phase, each is adsorbed to a greater or lesser extent relative to the other components of that mixture. Compounds that favor the stationary phase are held longer, and as a result move more slowly than do compounds that favor the mobile phase. It is these differences in equilibrium that allow for the separation of compounds in multi-component mixtures.

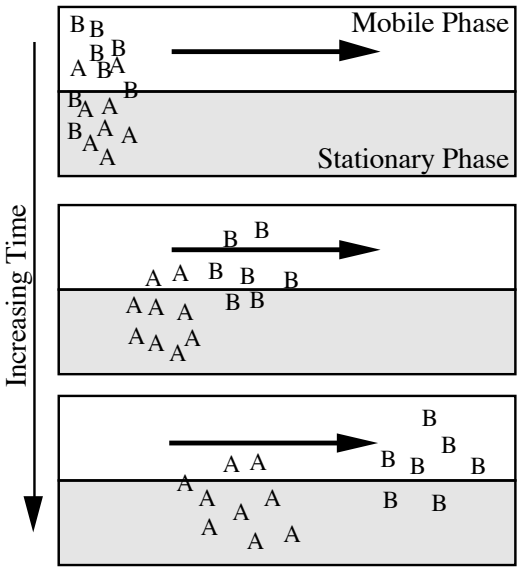


Figure 9: Chromatographic separation of a two-component mixture. As the mobile phase moves, it carries B faster than A because B "spends more time" in the mobile phase.

In the example shown here (Figure 9),

compound A interacts more strongly with the stationary phase than does compound B. As a result, more of A is adsorbed on the stationary phase at any given time. It follows that B thus spends more time in the mobile phase than does A, and is carried more quickly in the direction of the flow. As such, these two compounds will eventually separate, and the degree of separation will increase the longer the mobile phase travels.

The effectiveness of a mobile phase in eluting a compound along a stationary phase depends on the polarity of the adsorbent (stationary phase), the sample and the solvent (mobile phase). Generally speaking, the more polar the sample compound, the more tightly it will be bound to the stationary phase. Conversely, the more polar the solid phase, the more tightly it will bind a compound in the mobile phase. The order in which compounds will be eluted from silica or alumina is the reverse of the compounds ability to bind to the adsorbent. Binding strength increases with substrate polarity as shown in Table 1.

	Compound Type
Least Polar	Alkanes
	Alkenes
	Ethers
	Alkyl Halides
	Aromatics
	Aldehydes and Ketones
	Alcohols
	Amines
	Organic Acids
Most Polar	Salts

Table 1: Relative Binding Strengths of Common Organic Compounds.

	Solvent
Least Polar	Hexanes
	Carbon Tetrachloride
	Toluene
	Chloroform
	Diethyl Ether
	Ethyl Acetate
	Acetone
	Methanol
	Acetic Acid
Most Polar	Water

Table 2: Eluting Power of Some Chromatography Solvents on Silica.

The eluting power of various organic solvents parallels this order. Thus, the greater the polarity of the solvent, the greater its ability to dislodge and displace a polar compound from the stationary surface, and the faster the compound will move along. This property of solvents has quantified somewhat by listing them in order of their ability to displace solutes from adsorbents. This listing is know as an "*eluotropic series*" and will vary somewhat from adsorbent to adsorbent. An eluotropic series for silica gel is shown in Table 2.

A. Thin Layer Chromatography

Thin-layer chromatography (TLC) is used primarily as an analytical technique to determine the purity of a compound, the status of an ongoing reaction, or as a preliminary means of identification. The sample is spotted near the bottom of a glass or plastic plate which is coated with a thin layer (hence the name) of dry adsorbent (Figure 10). The plate is then placed in a covered beaker or jar that contains a small amount of the appropriate solvent. The level of the solvent in the jar must be below the level of the sample spots, and the atmosphere in the jar should be saturated with solvent vapors. A filter paper is generally used to help with the saturation.

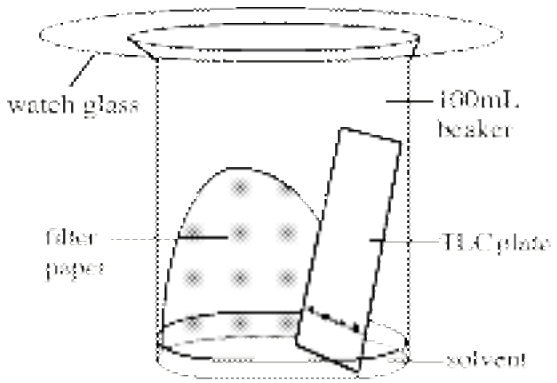


Figure 10: TLC developing chamber. Note that the spots are all above the level of solvent. This figure shows the TLC plate immediately after it is put into the chamber.

Capillary action draws the solvent up the plate. (If the jar is not saturated with solvent vapors, the solvent will not run all the way up the plate). When the *solvent front* is near the top of the plate, it is removed from the beaker and the location of the solvent front is marked with a pencil. If the compounds are colored, and the plate can be read easily, no other method of visualization is needed. If the compounds are not colored, they can be visualized using an ultra-violet lamp or a chemical stain, such as iodine.

For each spot on the TLC plate a characteristic value called the ratio to front, or R_f value can be calculated (Figure 11). R_f is defined as the ratio of the distance traveled by a spot (measured from the center of the spot) to the distance traveled by the solvent:

$$R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}$$

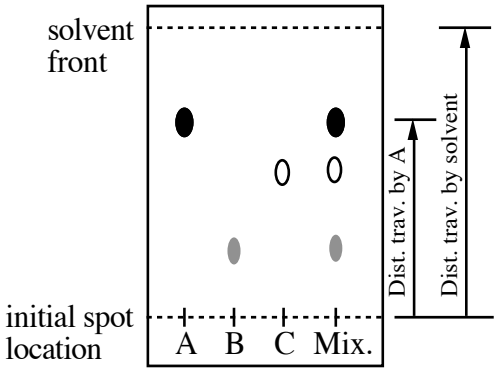


Figure 11: TLC plate after developing and staining. Note that the distances are measured from the initial spot location, not from the bottom of the plate.

Although the R_f is characteristic for a given compound, it depends greatly on the solvent and the type of adsorbent used. Consequently there are no tables of R_f values in the chemical literature. However, under standard conditions R_f can be used to identify the components of a mixture, to determine the purity of a compound, and as an indicator as to whether or not a reaction has gone to completion.

The difference in R_f values between two spots on a plate, ΔR_f, will also vary with the solvent, and is used as a measure of the performance of the separation. The choice of developing solvent is crucial. With too polar a solvent, all of the 'spots' will run to the top of the plate, and ΔR_f will be zero. With a very nonpolar solvent, the spots will not move from the baseline, and again $DR_f = 0$. Oftentimes, mixtures are used to adjust the polarity of the developing solvent, in order to achieve good separation. The information gained by TLC in terms of the relationship between solvent polarity and separation is also quite useful when choosing a solvent for column chromatography.

B. Column Chromatography

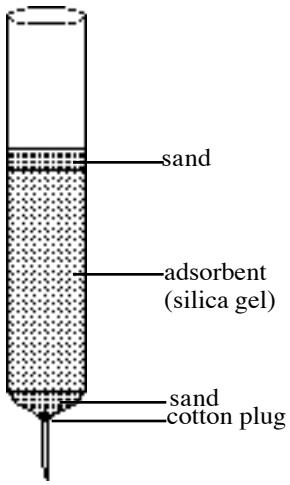


Figure 12: Column setup for chromatography.

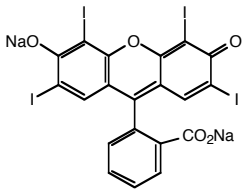
Column chromatography is perhaps the most frequently used method of purification in an organic research laboratory. It is governed by the same principles as thin-layer chromatography, except that the adsorbent or stationary phase is packed in a glass tube or *column*, rather than spread on a thin plate (Figure 12). A column may be packed 'wet' by pouring a solvent-adsorbent slurry into the tube, or 'dry' by filling it with dry adsorbent and then adding the solvent. The mixture to be purified is then dissolved in a small amount of the appropriate solvent and added carefully at the top of the column, so as not to disturb the packing. In rare cases, a solid mixture may be added directly. The column is *developed* by adding additional solvent to the top and collecting fractions of the *eluate* that comes out of the bottom. These fractions are then analyzed by TLC. For 'flash' column chromatography, moderate air pressure is used to push the solvent through the column.

As with thin-layer chromatography, the choice of solvent system is crucial for good separation, and the best separation is often achieved by using solvent mixtures. A single solvent mixture may be used to develop the column, or, in cases where the components to be separated have very different polarities, a solvent system which gradually increases in polarity (a polarity *gradient*) may be utilized. For example, a column may be developed first with a low-polarity solvent, such as hexane, and as fractions are collected the developing solvent is changed to 10:1, 5:1, and 1:1 hexane:ethyl acetate. As a rule of thumb, a good solvent system for column chromatography is one in which the least polar compound in a crude reaction mixture has an R_f value of 0.3 by TLC.

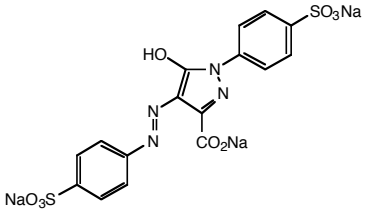
Procedure

A. Identification of Commercial Food Dye Components by Thin Layer Chromatography

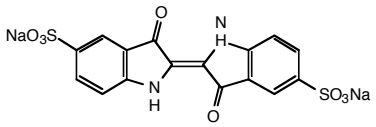
In this portion of the experiment you will be investigating the makeup of some common dyes used in commercial food colorings. You will first examine the commercial solutions and identify the number of components in each. Next you will identify these components. Some of the most widely used food dyes are shown below:



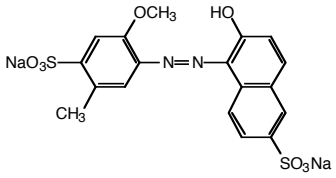
F,D&C Red No. 3
(Erythrosine)



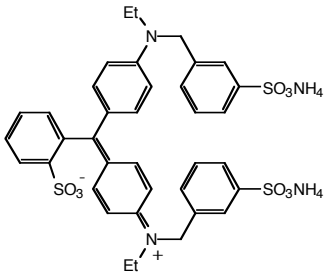
F,D&C Yellow No. 5
(Tartrazine)



F,D&C Blue No. 2
(Indigo Carmine)



F,D&C Red No. 40
(Allura Red)



F,D&C Blue No. 1
(Brilliant Blue FCF)

Draw a light pencil line about 1 cm from the bottom of a thin-layer chromatography (TLC) plate. Mark four equally spaced points along this line and label them A, B, C, and D (one for each solution of commercial food color). Using a micropipet, put a small spot of solution at the appropriate mark. *Remember that the best results are obtained from small, compact spots*.

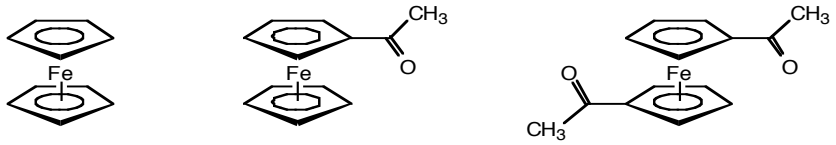
While the spots are drying, prepare a TLC developing chamber as follows: Fold and tear a filter paper in half and place it in a 100mL beaker. Obtain about 8mL of the prepared 3:1 2-propanol:ammonium hydroxide solution from the hood and transfer it to the beaker. The solvent should cover the bottom of the beaker to about 0.5 cm. Get the filter paper soaked with solvent. This filter paper acts as a wick to keep the developing chamber saturated with solvent vapors.

Once the spots have dried, carefully place the TLC plate in the beaker. IMPORTANT NOTE: To ensure even movement of the solvent, *the TLC plate should not touch the filter paper*. Watch the solvent move up the plate. This may take some time. When the solvent front is about 0.5 cm from the top of the plate, remove it from the developing chamber and immediately mark the location of the solvent front with a pencil. Measure and record the distances traveled by the solvent front as well as the distances from the origin to the center of each resolved spot. Be sure to also record a copy of your TLC plate. Calculate the R_f value for each spot.

Run a second TLC as above, this time using the (five) known F, D, & C dye solutions. Compare the R_f values of the known dyes with those of the commercial dye solutions. What components can you identify?

B. Separation of a Ferrocene Mixture by Column Chromatography

In this portion of the experiment you will be given a 3-component mixture containing ferrocene, acetyl ferrocene and 1,1'-diacetyl ferrocene. You will use column chromatography to separate this mixture into its individual components.



Ferrocene Acetylferrocene 1,1'-Diacetylferrocene

Prepare your column as shown in Figure 12. Take a small wisp of cotton and carefully push it into the narrow part of a Pasteur pipet. Clamp the pipet securely, point down. Add approximately 1/4 inch of sand, then 2 to 2.5 inches of silica gel. Finally, add 1/4 inch of sand above the gel.

Obtain a sample vial of the ferrocene, acetyl ferrocene, 1,1'-diacetyl ferrocene mixture. Add this mixture to the top of the column and cover it with a little more sand. Carefully add petroleum ether to the top of the column, and allow it to flow through. Collect the eluate in a small test tube. To make the solvent flow more rapidly, you will want to squeeze *gently* with a pipet bulb on the top of the column. IMPORTANT NOTE: Be careful not to let the liquid drop below the level of the silica gel, or to allow the silica to be sucked up by the pipet bulb. Either of these occurrences will damage your results. Keep adding petroleum ether until the first compound (yellow) has completely left the column. Use as many test tubes as are necessary to collect the eluate. Next, obtain about 10mL of 20% ethyl acetate/petroleum ether solution. Add this to the top of the column, and collect the second component. Be sure to use a new test tube as you will be collecting a new compound! Continue until the second compound has been eluted. Finally, collect the third compound using a 50% mixture of ethyl acetate in petroleum ether.

Once all components have been recovered, prepare a solution of the original mixture using any sample remaining in the vial. Label a TLC plate with seven "lanes": standards consisting of known pure samples of each of the components of the mixture, initial mixture, and each of the fractions (1, 2, and 3 - or more if necessary) that you collected. Use a capillary tube to spot the TLC plate with original solution and the three separate fractions from the column chromatography. Again, small, compact spots give the best results. Develop your plate in a developing chamber, prepared as in part A, using a 20% ethyl acetate/petroleum ether solution as the developing solvent. Allow sufficient time for the solvent to evaporate, and then visualize the plate first using UV light, then in an I_2 chamber (note the order). Circle the spots with a pencil, using a solid line for the UV visualization and a dashed line for the I_2. Record this information in your notebook. You should be able to tell if your chromatography was a success by the number of spots in each "lane". Identify the spots observed, and calculate their R_f values.

Results and Discussion

Discuss your results, and calculate the R_f value of each component you analyzed by TLC. In addition, for Part A, identify the components of the commercial food dyes by comparison of their R_f values with those of the F, D, & C Dyes. Be sure to clearly explain how and why you reached these conclusions. For Part B, note the order in which the compounds came off the column, and identify the components of the mixture. Discuss the relationship between binding strength and R_f value as it applies to your results. Again, be sure to clearly explain how and why you reached these conclusions.

Include a critical evaluation of this experiment as a separate page at the end of your report (after the Appendices). Suggestions for improvement are the most helpful, but you may comment on any aspect of this experiment, the written materials, etc. Your comments will not be graded, but the evaluation must be present if you are to receive full credit for this report. This page will be removed and not returned.

Fill out the attached data sheet and submit it to your TA when you turn in your report. This sheet will be graded separately from your report and will not be returned. As such, you will also need to incorporate the data presented here within the body of your report.

References

Abrams, C. "Organic Chemistry Laboratory", 2nd ed. Columbia University, Department of Chemistry: New York, 1992, pp 13-20.

Zubrick, J.W. "The Organic Chem Lab Survival Manual: A Student's Guide to Techniques", 4th edition, John Wiley and Sons: New York, 1997, pp 251-254.

Harwood, L.M.; Moody, C.J. "Experimental Organic Chemistry: Principles and Practice"; Blackwell Scientific Publications: Oxford, England, 1989, pp 155-159.

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Part 3: Separation of a Three Component Mixture by Extraction

Background

Extraction is one of the most commonly used techniques in organic chemistry. Natural products are frequently isolated from plant or bacterial sources by extraction, organic reactions are often followed by workups that employ extraction as part of the purification process, and extraction is particularly useful in the separation of acidic and/or basic components from an organic mixture.

The success of this method depends on the different solubilities of compounds in various solvents. Thus, an organic compound in the presence of two immiscible solvents, generally water and an organic solvent, will distribute (partition) itself between the two phases until equilibrium is reached. At equilibrium the ratio of concentrations of the solute in each layer is constant, and may be defined as the *distribution coefficient*, K:

$$\text{Distribution Coefficient (K)} = \frac{[\text{X}] \text{ in solvent B}}{[\text{X}] \text{ in solvent A}} \approx \frac{\text{solubility of X in solvent B}}{\text{solubility of X in solvent A}}$$

where $[X]_B$ is the concentration of solute in solvent B (generally the organic phase) and $[X]_A$ is the concentration of solute in solvent A (generally the aqueous phase). This relationship is independent of the total concentration of the solute and the actual amounts of the two solvents mixed. The distribution coefficient has a constant value for each solute considered and is dependent on the nature of the solvents used in each case.

Given that the distribution coefficient represents an equilibrium, it should be noted that not all the solute will be transferred (e.g. from solvent A to solvent B) in a single extraction unless the distribution coefficient, K, is very large ($K \approx 100$). In most cases multiple successive extractions with a small amount of solvent are required, and are generally more efficient than a single extraction with a larger amount of solvent. Generally speaking, any organic compound with K greater than 1 can be efficiently extracted from aqueous solution.

In cases where $K < 1$, a simple extraction process will not give a satisfactory recovery of organic solute from an aqueous solution. However, in this case the distribution coefficient can be altered by adding an inorganic salt such as sodium chloride to the aqueous layer. Since organic compounds are generally less soluble in a saturated salt solution than in water, the addition of NaCl shifts the equilibrium of solute between the two phases toward the organic layer, thereby increasing the distribution coefficient, and increasing the efficiency of extraction. This process is termed *salting out*. Conversely, in this situation, water tends to move into the saturated salt (brine) layer to help solvate the inorganic ions. Thus, saturated aqueous salt solutions are frequently used as preliminary drying agents to extract water molecules from the organic layer into the aqueous.

A. Purification of Organic Compounds

Extraction is an important tool for the preliminary purification of organic compounds. In this way, the desired product of a reaction may be separated from unreacted starting materials, unwanted by-products and impurities during an extractive workup of the reaction mixture. In a typical extraction sequence, water is frequently utilized as an extraction solvent to remove unwanted water soluble impurities (inorganic salts, polar, low molecular weight organics, etc.) from the organic reaction medium. As the solvent ability of water can be dramatically altered during the course of an extraction sequence by changing the pH of the aqueous layer, acidic or basic impurities that might otherwise remain in the organic layer can also be removed by formation of their corresponding water soluble salts (Figure 13). For example, an acidic impurity (AH), in the presence of base, forms a charged species, a *salt*, (A^- M^+) upon treatment with base thereby increasing its solubility in the aqueous layer, and effectively separating it from the uncharged components in the organic layer. A like procedure can be applied for the removal of basic impurities upon treatment with dilute acid.

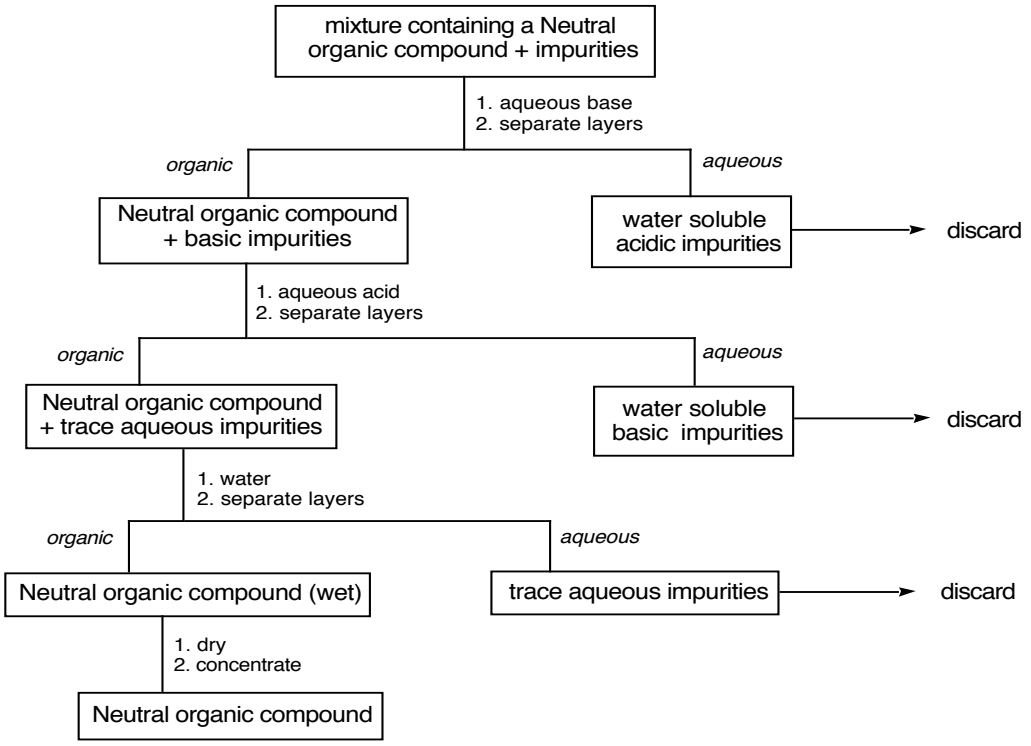

Figure 13: A general extraction sequence for the removal of acidic and basic impurities from a neutral organic compound.

B. Separation of Organic Compounds

As with the removal of various impurities, the acidic and basic properties of various organic compounds can be utilized as a mechanism for separating the components of a mixture. Organic

acids (carboxylic acids and phenols) and organic bases (amines) can be readily separated from each other and from neutral compounds by a careful extraction protocol (Figure 14). Thus, strong organic acids such as carboxylic acids ($pK_a \sim 5$) are easily converted into their sodium salts by reaction with weakly basic sodium bicarbonate. Weaker organic acids such as phenols ($pK_a \sim 10$) require a stronger base such as sodium hydroxide. These differences in acidity allow the selective separation of these various organic acids by extraction. Thus for an organic mixture containing both the carboxylic acid and a phenol, sodium hydroxide (a strong base) will react with both components; extracting both the carboxylic acid and phenol sodium salts into the aqueous medium. On the other hand, extraction with sodium bicarbonate will result in formation of only the carboxylic acid salt. As such, the carboxylic acid component will be drawn into the aqueous layer leaving the uncharged phenol in the organic layer, thereby allowing for an efficient separation of the two components. Conversely, organic bases such as amines are converted into water soluble hydrochloride salts by reaction with hydrochloric acid. Once separation has been effected, the various components can be isolated: from the organic layer by removal of the solvent; from the aqueous layer by neutralization and subsequent filtration or extraction. Using these principles it is possible to separate the various components from rather complex organic mixtures.

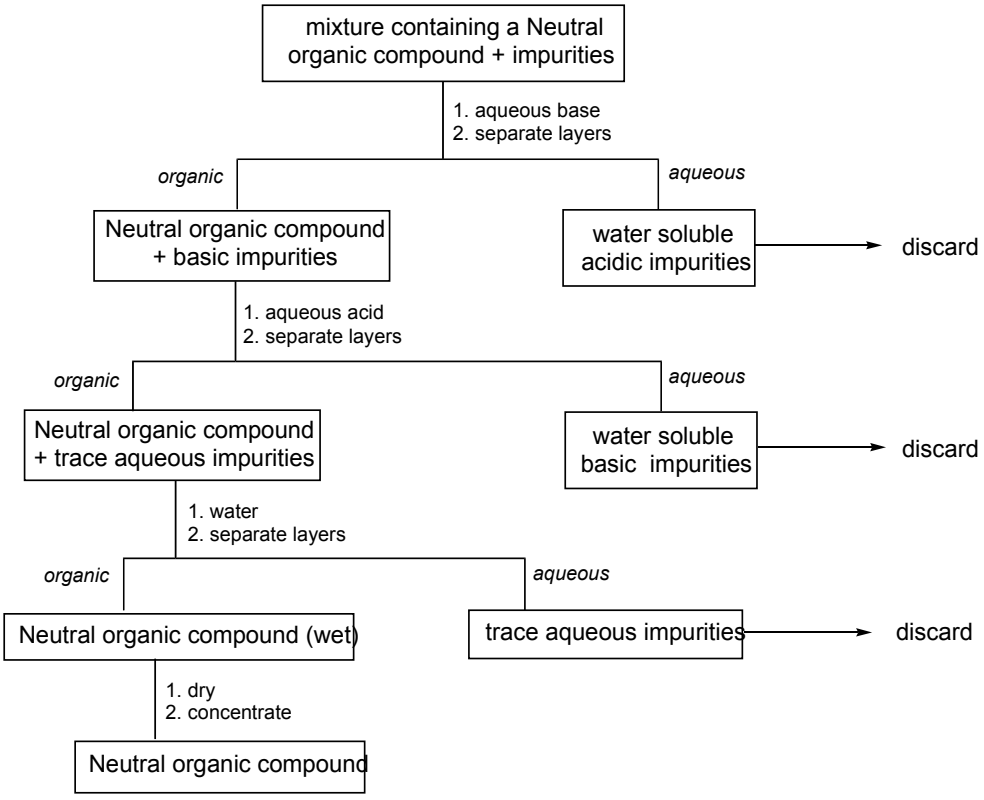

Figure 14: General approach to the separation of Neutral (N), Acidic (AH), and Basic (B) components of a mixture.

In this experiment, you will use the differences in acid strengths of carboxylic acids, phenols, and amides to separate *p*-toluic acid, *p-tert*-butylphenol and acetanilide from one another. First, you will extract only *p*-toluic acid into a $NaHCO_3$ solution. Then, you will extract *p-tert*-butylphenol into a NaOH solution. The neutral acetanilide will remain dissolved in the neutral organic solvent, *tert*-butyl methyl ether. Next, you will add HCl to each of the extracts to precipitate the water-insoluble p-toluic acid and *p-tert*-butylphenol. You will isolate the precipitates from the solutions by vacuum filtration and then air dry them. The third compound, acetanilide, will not react with either NaOH or $NaHCO_3$ and remains dissolved in the nonpolar organic solvent, *tert*-butyl methyl ether. To recover the acetanilide, you will dry the nonpolar layer with anhydrous magnesium sulfate ($MgSO_4$) and remove the excess solvent *via* rotary evaporation. The acetanilide can be crystallized by placing it in an ice bath.

After all three of the compounds are dry, you will measure the mass of each isolated compound. Next, you will measure the melting point of each compound and assess its purity by comparing the experimentally measured melting point with the literature value. Also, you will collect IR spectra for *p*-toluic acid and *p-tert*-butylphenol. Finally, you will recrystallize the *p*-toluic acid from ethanol. Your recrystallized product can then be compared with the crude *p*-toluic acid to assess the purity of the recrystallized product.

NOTE: An extremely important aspect of separations and extractions using a separatory funnel is the correct identification of the organic and aqueous layers. The easiest and most accurate way to predict which layer will be on top and which layer will be on bottom is to compare the densities of the two solvents being used. The denser layer will generally be the bottom layer, and the less dense layer will sit on top of it. Aqueous layers are generally (but not always) more dense than organic layers (notable exceptions are halogenated solvents, like chloroform and dichloromethane). You should have already obtained the densities of the solvents during your prelab preparation, so make sure that you have the carbon copy of your prelab available during the lab period! The density of an aqueous solution (e.g. 0.5 M aqueous $NaHCO_3$) is typically approximated by the density of pure water (1 g/mL). To confirm your prediction, add a small portion (1-2 mL) of the second solvent to the separatory funnel and take note of where the new, small layer forms.

Ideally, the product that you wish to isolate will be found entirely in one layer, but if this layer is incorrectly identified and discarded, you may have to restart the experiment. Therefore, **it is a good idea to save all the layers of any extraction until the product crystals are isolated. This way, if a mistake was made, it can be corrected.**

Procedure

Weigh out 2.0-2.75 g of the mixture of *p*-toluic acid, *p-tert*-butylphenol, and acetanilide provided by your TA into a 100-mL beaker. Assume this mixture is made up of a 1: 1: 1 ratio of *p*-toluic acid, *p-tert*-butylphenol, and acetanilide by weight. Dissolve the mixture in 25 mL of *tert*-butyl methyl ether. Stir the mixture to ensure complete dissolution. Pour this solution into a 125-mL separatory funnel supported by an iron ring attached to the lab racks. **Important reminder:**

Be sure the stopcock is closed on the separatory funnel, and that there is a clean beaker below the funnel to catch any of the solution that drips through.

p-Toluic Acid Extraction

Slowly add 10 mL of 0.5 M aqueous $NaHCO_3$ to the ether solution in the separatory funnel. Take note of whether the new aqueous layer forms above or below the organic (tert-butyl methyl ether) solution. **Caution: the reaction of sodium hydrogen carbonate ($NaHCO_3$) with a carboxylic acid produces carbon dioxide (CO_2) gas, which can result in foaming.** Place the stopper in the top of the separatory funnel, and invert the funnel while holding the stopper in place. Gently mix the two layers by rocking and shaking the separatory funnel back and forth.

With the separatory funnel inverted, open the stopcock to vent any gas that is generated. Listen for the gas as it exits through the stopcock. Continue this mixing process, gradually increasing the force of the mixing, until the funnel can be shaken quite vigorously with no gas being produced upon venting. Place the funnel in the iron ring and allow the layers to separate.

Remove the stopper from the top of the funnel, and open the stopcock to allow the aqueous layer to drain into a clean, labeled 100-mL beaker. *Note*: if you open the stopcock with the stopper in the top of the funnel, a slight vacuum will be created, and the bottom layer will not drain from the funnel. When the interface between the layers just reaches the bottom of the funnel (top of the stopcock), close the stopcock to retain the ether layer in the funnel.

Add a second 10 mL of 0.5 M aqueous $NaHCO_3$ to the funnel to remove any *p*-toluic acid remaining in the ether layer. Mix with frequent venting. After the layers have separated, drain the aqueous layer into the beaker with the first extract. Repeat with a third 10 mL 0. 5 M aqueous $NaHCO_3$ portion.

Add 5 mL of distilled water to the separatory funnel and mix. Drain the water layer into the beaker containing the three $NaHCO_3$ solution extracts. Place this beaker aside for now.

p-tert-Butylphenol Extraction

Add 10 mL of 0.5 M NaOH to the ether solution remaining in the separatory funnel. Mix the layers as before so that the NaOH and the *p-tert*-butylphenol can react. Remember to mix cautiously at first with frequent venting through the stopcock. Allow the layers to separate, and drain the aqueous NaOH layer into a clean, labeled 100-mL beaker.

Repeat the extraction of the ether layer with a second 10 mL of 0.5 M NaOH solution. Drain the NaOH layer from the separatory funnel into the 100-mL beaker containing the first NaOH extract. Repeat with a third 10 mL of 0.5 M NaOH.

Add 5 mL of distilled water to the ether remaining in the separatory funnel and mix. Allow the layers to separate. Drain the water layer into the 100-mL beaker containing the three NaOH extracts. Save the NaOH extracts for the isolation of the *p-tert*-butylphenol.

NOTE: These isolations may be done in any order. Some of you should isolate the acetanilide first, so that you can get on the rotary evaporators as quickly as possible. Those of you who do not get on the rotovaps right away may do the other isolations while you are waiting. This will avoid long, unproductive waits for the rotovaps.

p-Toluic Acid Isolation

Select the 100-mL beaker containing the $NaHCO_3$ extracts. To precipitate the *p*-toluic acid, carefully add 6 M HCl drop wise to the $NaHCO_3$ solution. Notice that foaming occurs, and a precipitate of *p*-toluic acid forms. Continue to add the 6 M HCl, drop wise with stirring, until no more solid is produced and the solution tests acidic (pH <3). To test for acidity, remove a drop of the solution with a stirring rod and place the drop on a piece of pH test paper.

Weigh a filter paper and record its mass. Using the weighed filter paper, separate the crystals from the solution using vacuum filtration with a Büchner funnel. Support the crystals and paper on a watch glass and allow the crystals to air dry. When the crystals are dry, weigh them and calculate a percent recovery of *p*-toluic acid. Measure the melting point of the crystals using a Mel-Temp apparatus. Obtain an IR spectrum of your *p*-toluic acid (use mineral oil; be sure to compare with a mineral oil standard).

p-tert-Butylphenol Isolation

Select the 100-mL beaker containing the NaOH extracts. To remove any remaining traces of *tert*-butyl methyl ether that might inhibit the crystallization of the phenol, heat the NaOH solution to about 60^0C on a hot plate in a ***fume hood.*** Remove the beaker from the hot plate and allow the solution to cool.

To precipitate crystals of *p-tert*-butylphenol, carefully add 3 M HCl drop wise to the cooled solution until it is acidic. If the phenol separates as an oil, cool the mixture in an ice bath to facilitate crystallization.

Weigh a filter paper and record its mass. Using the weighed filter paper, separate the *p-tert*-butylphenol crystals from the solution by filtration with a Büchner funnel. Support the crystals and paper on a watch glass and allow the crystals to air dry. When the crystals are dry, weigh them and calculate a percent recovery of *p-tert*-butylphenol. Measure the melting point of the crystals using a Mel-Temp apparatus. Obtain an IR spectrum of your *p-tert*-butylphenol (use mineral oil; be sure to compare with a mineral oil standard).

Acetanilide Isolation

Select the separatory funnel containing the ether layer. Transfer the ether-acetanilide solution from the separatory funnel to a clean 125-mL Erlenmeyer flask. Add approximately 0.75 g of anhydrous $MgSO_4$ to the flask to remove any traces of water from the solution. Stopper the flask and allow it to stand for 5 min. with occasional swirling. After the anhydrous $MgSO_4$ has adsorbed water, it will be clumpy and will not swirl around like snow in a Christmas paperweight.

Preweigh a 100-mL round-bottomed flask and record its weight. Filter the dried ether-acetanilide solution into the 100-ml, round-bottomed flask using a fluted filter paper. Remove the solvent using a rotary evaporator. Your TA will show you how the rotary evaporator works. If the acetanilide does not crystallize while the flask is attached to the rotovap, but no more solvent is being removed, remove the oil residue from the rotovap. Crystallize the oil residue (the acetanilide) by cooling the round-bottomed flask in an ice bath. If necessary, scratch the bottom of the flask with a glass rod, or add a seed crystal, to induce crystallization. Allow the acetanilide crystals to dry. When the crystals are dry, weigh them and calculate a percent recovery of acetanilide. Measure the melting point of the crystals using a Mel-Temp apparatus.

***p*-Toluic Acid Recrystallization**

Weigh approximately 0.5 g of the crude *p*-toluic acid into a large test tube. Add warm ethanol drop wise to the product while heating it in a hot water bath. Shake the test tube often to help dissolve the *p*-toluic acid. You should use the minimum amount of solvent necessary to dissolve your crude compound. Too much solvent will markedly reduce the recovery of your *p*-toluic acid.

Once the *p*-toluic acid has dissolved, allow the solution to cool slowly to room temperature. Do not shake or stir the mixture while crystals are forming. Do not touch it to a cool surface. When crystallization is complete at room temperature, cool the mixture further on an ice bath. Collect your *p*-toluic acid by vacuum filtration using a Büchner funnel and a weighed filter paper, and wash it with about 1 mL, of ice cold EtOH. Scrape the solids gently while filtering to help them dry more quickly.

Once filtration is complete, continue to apply vacuum until the crystals are dry. Record the weight of your recrystallized *p*-toluic acid, and calculate the percent recovery**.** Obtain a melting point of the recrystallized *p*-toluic acid, and compare this melting point range to the crude melting point range.

Preparation of NMR Sample for Experiment 3: NMR

At the end of this experiment, your TA will show you how to prepare a sample for NMR analysis. You will then be asked to prepare an NMR sample of a known compound and submit it to your TA. You will use the NMR spectrum that you will receive to complete question #7 of the Expt 3 assignment.

Results and Discussion

In your results and discussion section you should discuss several things. First, you should make a statement as to how well the separation worked based on percent recovery of the three components. Second, you should make a statement as to how well the separation worked based on melting points. You should also discuss this in terms of the IR spectra of *p*-toluic acid and *p-tert*-butylphenol. Were the spectra clean in both cases? You should also address the results of your recrystallization based on percent recovery and melting point comparisons. Discuss the purity of all compounds including the crude and recrystallized *p*-toluic acid. Your IR spectra should be included in Appendix B of your report. Submit your data sheet along with your report.

References

Harwood, L.M.; Moody, C.J. "Experimental Organic Chemistry: Principles and Practice"; Blackwell Scientific Publications: Oxford, England, 1989, pp 114-127.

Zubrick, J.W. "The Organic Chem Lab Survival Manual: A Student's Guide to Techniques", 4th ed. John Wiley and Sons: New York, 1997, pp 121-138.

Loudon, G.M. "Organic Chemistry", 2nd ed.; Benjamin/Cummings: Menlo Park, CA, 1988, pp 454-467

Name: _____ Section: _____

ID #: _____ Date: _____

Part 1 Distillation, Microboiling points and Gas Chromatography (GC)

Distillation(s) you ran (simple or fractional): _____

Lab Partner: _____

Simple Distillation	Sample Volume (mL)	Boiling Range (°C)	Sample Composition (From GC Trace) % Hexanes	% Toluene	Volume Hexanes	Volume Toluene
Fraction A:	_____	_____	_____	_____	_____	_____
Fraction B:	_____	_____	_____	_____	_____	_____
Fraction C:	_____	_____	_____	_____	_____	_____
Residue	_____					
Total:	_____				_____	_____

% Recovery (Hexanes): _____

% Recovery (Toluene): _____

Fractional Distillation	Sample Volume (mL)	Boiling Range (°C)	Sample Composition (From GC Trace) % Hexanes	% Toluene	Volume Hexanes	Volume Toluene
Fraction A:	_____	_____	_____	_____	_____	_____
Fraction B:	_____	_____	_____	_____	_____	_____
Fraction C:	_____	_____	_____	_____	_____	_____
Residue	_____					
Total:	_____				_____	_____

% Recovery (Hexanes): _____

% Recovery (Toluene): _____

Micro Boiling Point: Unknown #_____ bp°C_____

Identity_____

Gas Chromatography:

Unknown # _____	Unknown Liquid	Retention Time
	_____	_____

Part 2: Column Chromatography and Thin Layer Chromatography (TLC)

Thin-Layer Chromatography:

	original dye color:	color of components:	R_f value:	F, D, & C Dye:
A	Yellow	_____	_____	_____
		_____	_____	_____
		_____	_____	_____
B	Red	_____	_____	_____
		_____	_____	_____
		_____	_____	_____
C	Blue	_____	_____	_____
		_____	_____	_____
		_____	_____	_____
D	Green	_____	_____	_____
		_____	_____	_____
		_____	_____	_____

Column Chromatography:

	component:	color:	R_f value:
1.	_____	_____	_____
2.	_____	_____	_____
3.	_____	_____	_____

Part 3: Extraction, Isolation and Recrystallization

Weight of 1:1:1 Mixture Used (g):_____

Weight of Isolated *p*-Toluic Acid (g):_____

Percent Recovery:_____

Melting Range of *p*-Toluic Acid (°C):_____

Weight of Isolated *p-tert*-Butylphenol (g):_____

Percent Recovery:_____

Melting Range of *p-tert*-Butylphenol (°C):_____

Weight of Isolated Acetanilide (g):_____

Percent Recovery:_____

Melting Range of Acetanilide (°C):_____

Weight of *p*-Toluic Acid Used for Recrystallization (g):_____

Weight of *p*-Toluic Acid Recovered from Recrystallization (g):_____

Recrystallization Percent Recovery:_____

Melting Range of
Recrystallized *p*-Toluic Acid (°C)_____