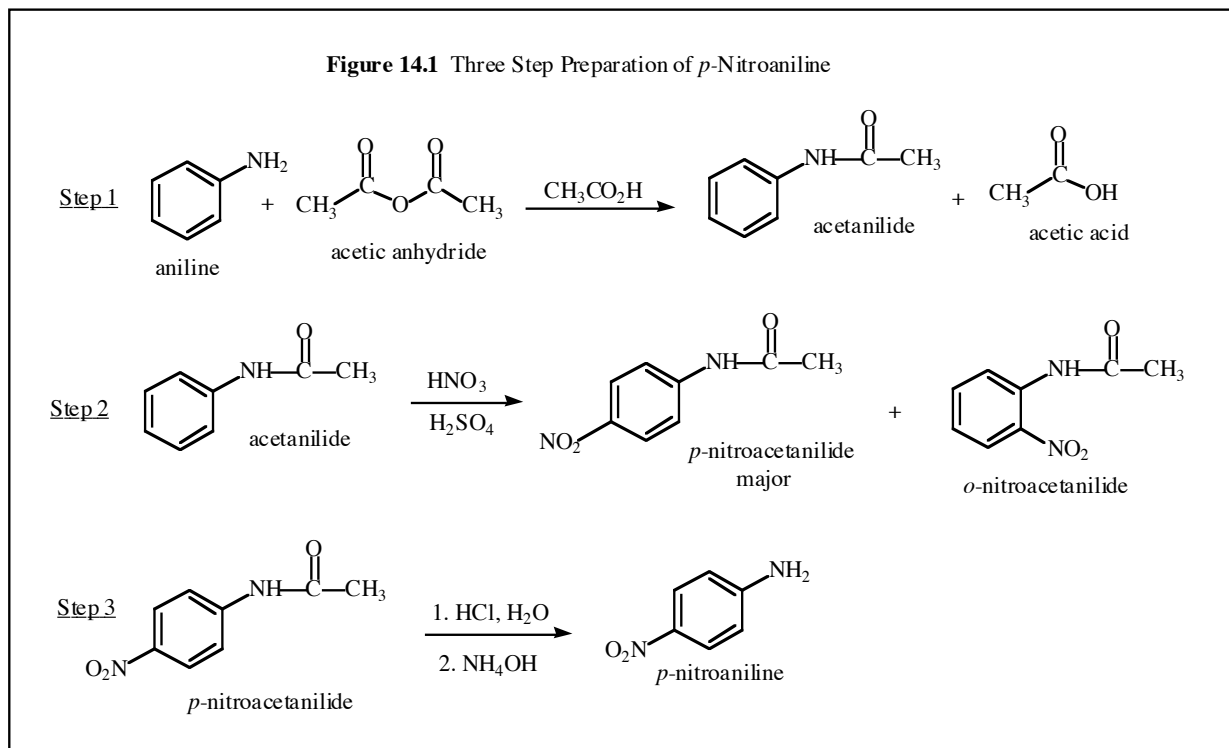


## Experiment 14

### Preparation of *p*-Nitroaniline

This experiment usually takes three weeks to complete and counts as two laboratory experiments (40 points). We will do a three-step synthesis to make *p*-nitroaniline from aniline and then we will characterize our product using the new and very useful technique of thin layer chromatography (TLC). The overall reaction scheme is given in Figure 14.1

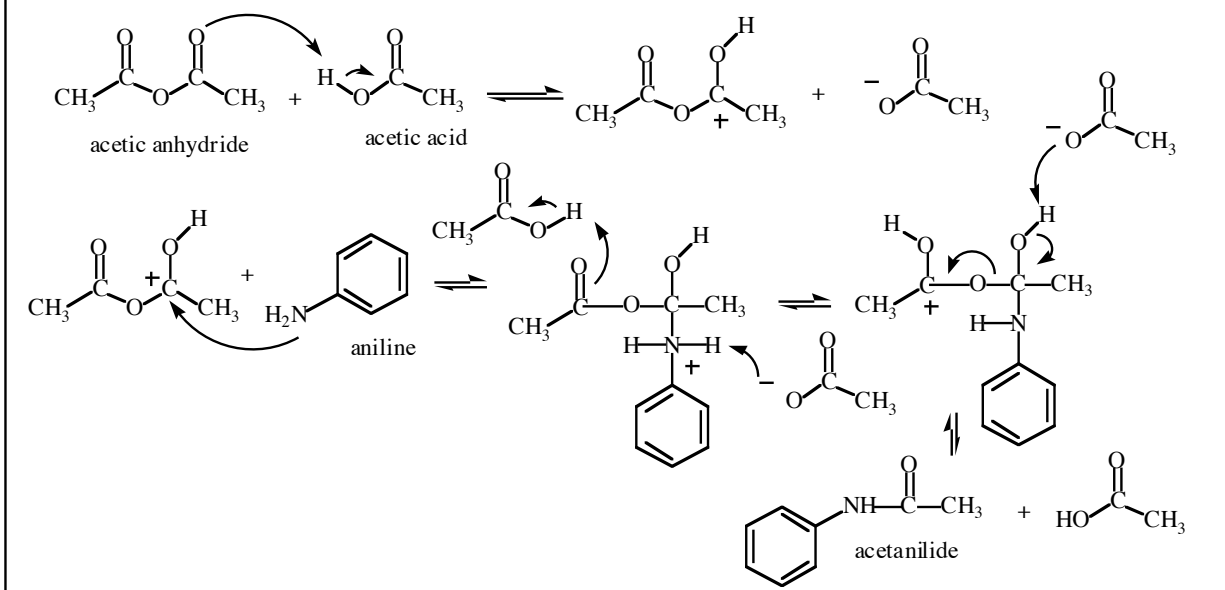


#### Step 1: Acetylation of Aniline

In the first step we need to put the removable acetyl protecting group on the nitrogen of aniline. The acetyl group is electron withdrawing and it therefore makes the lone pair on the nitrogen less reactive either in an oxidation reaction or a protonation reaction. The nitric acid used in the nitration step is a fairly strongly oxidizing agent and we could form an N-oxide. The nitration conditions (nitric acid and sulfuric acid) are strongly acidic. Protonation of the nitrogen of aniline makes it a very strong deactivating group, making the aromatic ring less susceptible to reaction with the nitrating agent and the  $\text{NH}_3^+$  group would be a *meta* director. Another value of the acetyl protecting group is that it is bulky group and preferentially directs the nitration to the *para* position rather than the *ortho* position.

The full mechanism for the reaction in Step 1 is given in Figure 14.2. Acetic anhydride is partially protonated by the acetic acid. This makes the anhydride an even better electrophile for the nucleophilic nitrogen of aniline. This attacks to form the tetrahedral intermediate, which, after proton transfer, loses acetic acid.

**Figure 14.2** Mechanism of Acetylation Reaction



### Physical Constants

Compound	Mol. Wt (g/mol)	Density (g/mL)	b.p. (°C)	m.p. (°C)
Aniline	93.13	1.022	184	-6
Acetic anhydride	102.09	1.082	138-142	-73
Acetic acid	60.05	1.049	116-117	15-16
Nitric acid (conc. 15.8 M)	63.01	1.420	-	-
Sulfuric acid (conc. 36 M)	98.09	1.840	330	-
Acetanilide	135.17	solid	-	113-115
<i>p</i> -nitroacetanilide	180.16	solid	-	215-217
<i>p</i> -nitroaniline	138.13	solid	-	147-148

### Procedure:

The set-up for your apparatus is shown in Figure 14.3. Note that there is NO STOPPER at the top of the condensing column. This is left open to the atmosphere so as to avoid a pressure build-up inside the flask when heating. Remember: NEVER HEAT A CLOSED SYSTEM.

Dissolve 4.0 mL of aniline in 10 mL of acetic acid in a 100 mL round bottom flask. To this solution, add 5 mL of acetic anhydride and mix well by swirling. CAUTION: the reaction is exothermic and the flask becomes warm. Add two boiling chips, attach a condensing column and attach the hoses for water cooling (water-in at the bottom and water-out at the top). Heat at a gentle reflux for fifteen minutes. You can use a heating mantle and rheostat or your Bunsen burner with a wire gauze pad.

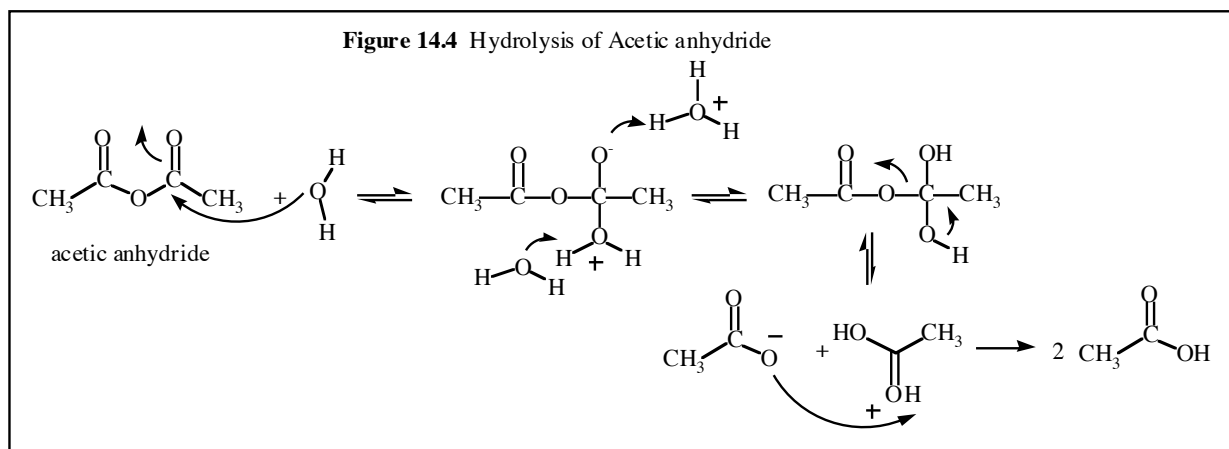
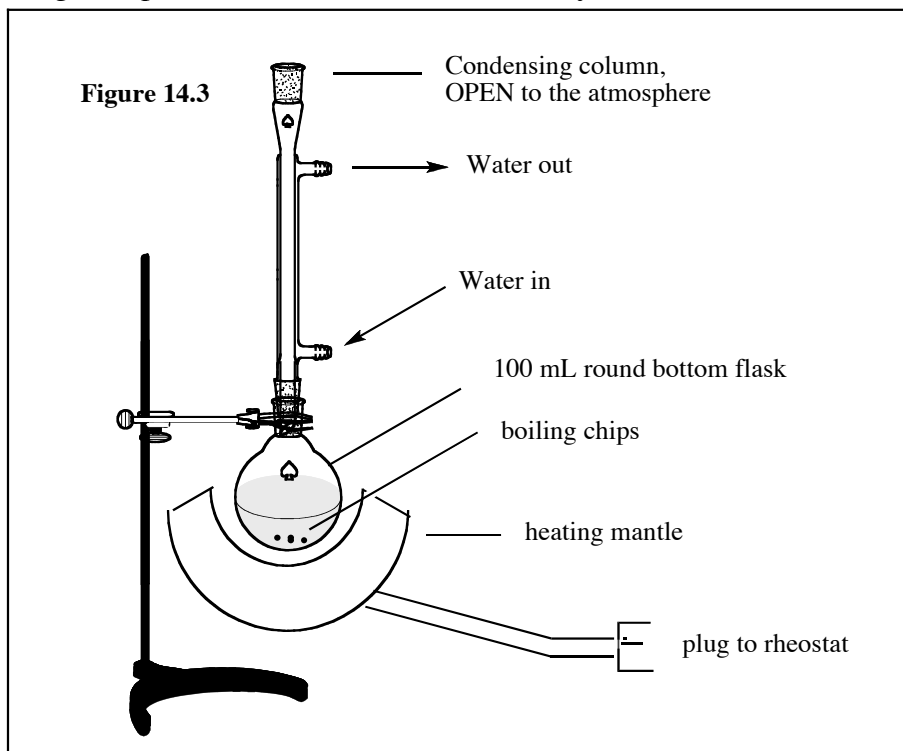
### Work up

After fifteen minutes, lower the iron ring under your heating mantle and allow the flask to cool slightly. CAUTIOUSLY add 5 mL of cold water through the top of the condenser into the reaction mixture. Boil the

solution for an additional five minutes so as to hydrolyze any unreacted acetic anhydride. The hydrolysis reaction is shown in Figure 14.4.

After boiling for five minutes, allow the reaction mixture to cool slightly and then pour it slowly with stirring into 30 mL of ice cold water. After allowing the mixture to stand for 15 minutes with occasional stirring, collect the precipitate by suction filtration using your Buchner funnel. Be sure to wet the filter paper before you do the filtration.

After the initial filtration, disconnect from the vacuum, wash the solid crystals with 10 mL of cold water and reconnect the vacuum tube for a couple of minutes more so as to dry the product as much as possible. Transfer the crystals to a watch glass and leave them to dry in your locker until the next laboratory session. Weigh the product and record the number in your notebook.



Take about 0.1 gram of your product and recrystallize it from water using your smallest test tube. Use the standard recrystallization technique described in Expt. 13 and elsewhere. Briefly, cover your solid with

solvent, heat the solvent to boiling, leaving a spatula in the test tube at all times; add more solvent in small increments, heating back to boiling with each new addition until the solution becomes clear. Once the solution becomes clear, remove it from the heat and allow it to cool slowly to room temperature and then immerse it in an ice bath. Collect the crystals by suction filtration using your Hirsh funnel. Leave the crystals to dry in your locker until the following lab period; then determine the melting point.

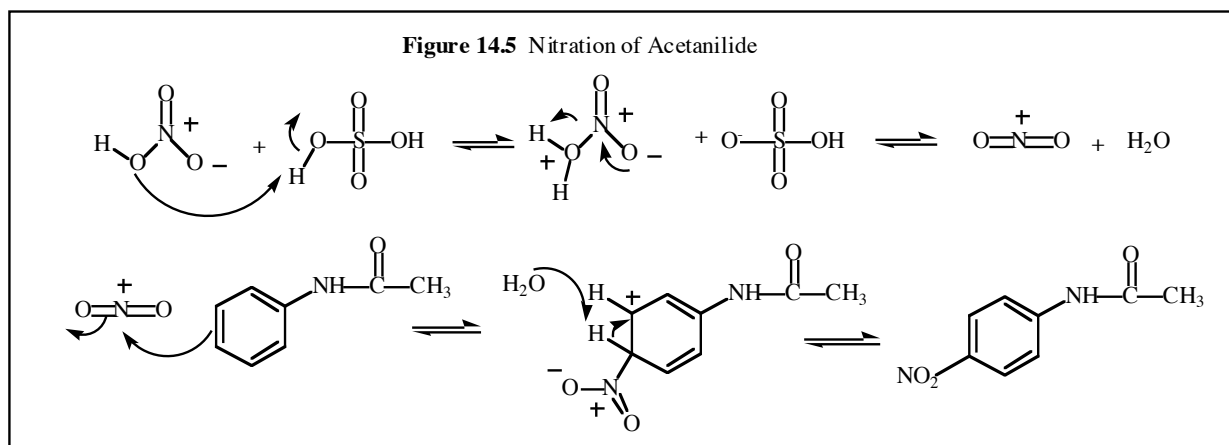
Save the rest of the product in your locker for the next step. Find the melting point range and calculate the percent yield for this first step. Hand in a Yield Report sheet.

## Step 2: Nitration of Acetanilide

In this step you first form the nitronium ion *in situ* by dehydration of nitric acid. Sulfuric acid is the dehydrating agent. The nitronium ion is a very powerful electrophile and will react with the  $\pi$ -electrons of the aromatic ring of aniline. These reactions are shown in Figure 14.5.

### Procedure:

In a 125 mL Erlenmeyer flask, dissolve 3.4 g of acetanilide in 4 mL glacial acetic acid. You may need to warm the solution gently in order to get all the solid material to dissolve. Use your Bunsen burner for this purpose. Stir with your glass stirring rod and pass the Erlenmeyer back and forth through a low flame. **DO NOT OVERHEAT.**



Add 5 mL of concentrated ICE COLD sulfuric acid. Add the sulfuric acid SLOWLY 1 mL at a time using a medicine dropper or pipette.

In a separate 25 mL Erlenmeyer flask, prepare a nitrating mixture by adding 1.8 mL of concentrated nitric acid (CAUTION!!) to 2.5 mL of ICE COLD sulfuric acid. Do this in an ice bath. Be careful. The addition reaction is exothermic. Add the nitric acid slowly using your medicine dropper. If you spill any acid on your hands wash them immediately. Cool the nitrating mixture to room temperature.

Add the nitrating mixture DROPWISE using a medicine dropper. Swirl the flask to mix the reagents thoroughly. Keep the temperature in the 20-25 °C range as you add the nitrating mixture. If the temperature gets too hot, cool the flask in the ice bath. Add 1-2 drops and swirl, monitoring the temperature carefully.

After all the nitrating mixture has been added, allow the solution to stand at room temperature for 40 minutes in order for the reaction to go to completion. **DO NOT LEAVE IT FOR MORE THAN ONE HOUR** or decomposition or a second nitration may start to occur.

Pour the reaction mixture slowly, with stirring, into a mixture of 50 mL of water and 10 g of ice.

Collect the product by suction filtration using your Buchner funnel. Press the solid firmly with your spatula to help remove as much liquid as possible. Since there is still a lot of the nitric/sulfuric acid mixture remaining, we need to wash the product thoroughly with water. To do this, transfer the solid filter cake to a

beaker containing 40 mL of ice cold water. Mix the suspension thoroughly to help wash off the acid and do another suction filtration using a new piece of filter paper in your Buchner funnel. Disconnect the filter flask from the vacuum and add an additional 10 mL cold water. Reconnect the vacuum and filter off this water.

Using your spatula, spread the material on a clean, dry watch glass and leave it to dry in your locker until the following lab period. Recrystallize a small sample – a spatula tip is enough – from 95% ethanol using a small test tube, a hot palate and hot water bath, and then take a melting point.

### **Step 3: Preparation of *p*-Nitroaniline**

Transfer all of the crude *p*-nitroacetanilide that was prepared above in Step 2 to a 100 mL round bottom flask. Add 10 mL of water and 10 mL of concentrated hydrochloric acid. Add two boiling chips and reflux the mixture at a gentle boil for 15-20 minutes using your heating mantle. Use the same set-up that you used in step one (see Figure 14.3). Since we are not using a flammable organic solvent, it is also safe in this step to use a low flame with your Bunsen burner, using your tripod and wire gauze. As you heat, swirl the flask to ensure mixing and to dissolve any remaining solid. All of the solid will dissolve and the solution will become orange in color.

After 20 minutes of reflux at a gentle boil, remove the heat source and add 15 mL of water. Cool the flask to room temperature. Crystals of *p*-nitroaniline may separate.

Prepare a solution containing 10 mL concentrated aqueous ammonia (CAUTION: STENCH!), 40 mL water and 25 g ice in a 400 mL beaker. Pour the solution of *p*-nitroaniline from above into this solution. Stir the suspension and test the pH of the solution by placing a drop on the test strip. It must be strongly basic (blue to litmus). If not, add more concentrated ammonia.

Collect the orange precipitate of *p*-nitroaniline by suction filtration using your Buchner funnel and wash the solid filter cake with 10 mL of cold water.

**Set aside a small, spatula tip of the crude product in a small test tube.** We will use this in the TLC analysis step.

Recrystallize the rest of the material from water using the instructions given in Step 1. It should take about 30 mL water per gram of product. You can get a crude weight of your material but remember that it is wet and the actual weight is less. Start with about 20 mL/g and heat the water to boiling. Then add more water as necessary, bringing the total solution to a boil after each addition of water.

After cooling the crystals in an ice bath, collect your product by suction filtration and dry it thoroughly. Record the weight and calculate the percent yield. Also take the melting point, drying a small sample on a small piece of filter paper. Turn in your product in a small vial along with your Organic Yield Report sheet.

The crude and recrystallized product will be analyzed by thin layer chromatography (TLC).

### **Thin Layer Chromatography**

#### **Background:**

We will use the technique of thin layer chromatography to analyze the purity of the crude *p*-nitroaniline and the crude material we just synthesized. TLC is a fast and very convenient technique for determining both the identity and purity of a compound.

In its simplest form, TLC is accomplished by spotting a small amount of the test substance, dissolved in an appropriate solvent, on a thin layer of absorbent which is backed by a glass or plastic plate (Figure 14.6). The plate is placed in a closed jar (developing chamber) that contains a small amount of solvent. It is also a good idea to put in a piece of folded filter paper to line the inside of the jar. This will help to saturate the atmosphere inside the developing container with solvent vapors.

As the solvent moves up the plate by capillary action, wetting the dry absorbent, the dissolved material (or materials) will move up the plate and “partition” itself between the absorbent and the solvent that is moving

up the plate. The distance the compound moves in a particular solvent system from the base of the plate (called the baseline) is a characteristic of the compound just like the melting point or boiling point. If we divide this distance by the distance the solvent moves, we get a number called the  $R_f$  value (or Retention Factor).

Though two compounds may have the same  $R_f$  value, if we spot an unknown compound against a known standard compound and the two compounds move the same distance up the plate, we can be fairly sure that these two compounds are the same.

Separation of the components in the test substance results from the difference in the rate at which the components of the mixture advance up the plate. In our experiment the absorbent that we will use is called silica gel. This is a polymer of silicon oxide (see Figure 14.7). The surface is quite polar since it is made up of OH groups capable of hydrogen bonding to polar compounds. Polar compounds tend to adhere to silica gel and move more slowly up the plate while less polar compounds will travel more rapidly up the plate and therefore move a greater distance.

The solvent is usually an organic solvent that has a similar polarity to the compounds that are being tested. Very often a mixture of solvents is used. In our experiment we will use methylene chloride, a moderately polar solvent. A very useful mixture is ethyl acetate and hexane. Solvent mixtures containing different proportions of the two solvents can be used. Hexane of course is non-polar and increasing proportions of ethyl acetate increase the polarity of the mixture.

If the components are colored, they may be observed directly as spots on the plate. Most organic compounds, however, are colorless. To visualize colorless spots, the plate can be exposed to iodine vapors, which react with many organic compounds to produce a brown stain. An iodine chamber can easily be made by placing several crystals of iodine and the TLC plate in a closed jar. In a few minutes, brownish spots will appear on the plate to mark the location of the organic compounds.

Another technique for visualizing non-colored spots is to expose them to ultraviolet light. Many organic compounds, particularly those with  $\pi$ -electrons will absorb UV light, thereby appearing dark when exposed to a UV lamp on a plate that is coated with a white absorbent.

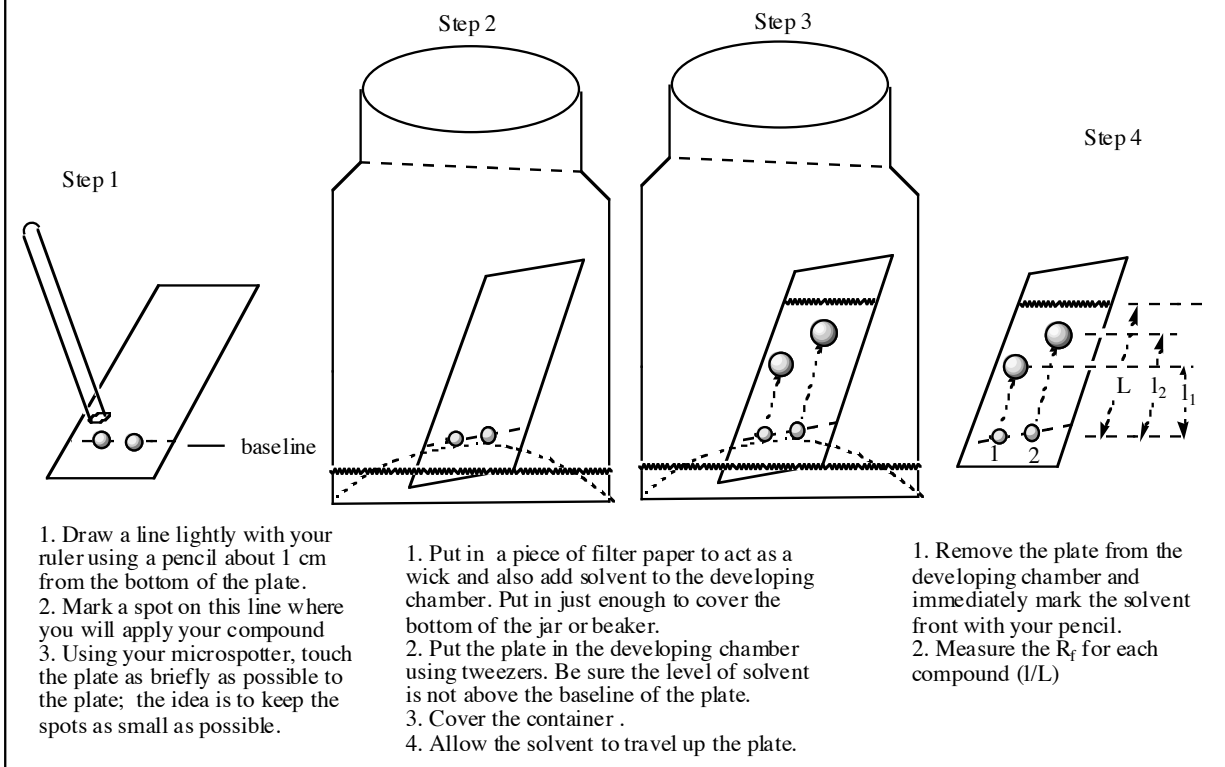
The number and location of the spots can be used to deduce information about the identity and purity of the sample. A pure substance will produce only one spot on the plate and a substance that is a mixture of compounds will have two or more spots.

We will find the  $R_f$  value (Retention factor). This is defined as the distance that the compound moves from the baseline to its final position on the plate ( $l_1$  or  $l_2$  in Figure 14.6) divided by the distance the solvent moves ( $L$  in Figure 14.6).

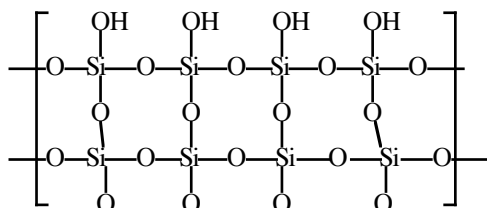
For this experiment, a practice TLC procedure using a mixture of organic dyes will first be carried out. The TLC plates and the enclosed jar (developing chamber) will be provided by the stock room. Put just enough methylene chloride in the jar to wet the liner and to cover the bottom of the jar. **YOU DO NOT WANT THE LEVEL OF SOLVENT TO BE ABOVE THE LEVEL OF THE SPOTS ON THE TLC PLATE.**

The idea is to keep the spots of compounds that you apply to the baseline as small as possible. In order to facilitate this, prepare several microcapillary pipettes from melting point capillaries as shown in Figure 14.8. The melting point capillary should be open at both ends. While twisting the capillary in your fingers, heat the center of the tube until it begins to soften. Then **SLOWLY** draw apart the ends in a continuous motion so that you stretch out the glass. Break the capillary into two pieces. Be careful. The ends of the capillary can be very sharp.

**Figure 14.6** Performing a TLC Analysis



**Figure 14.7** Structure of Silica Gel

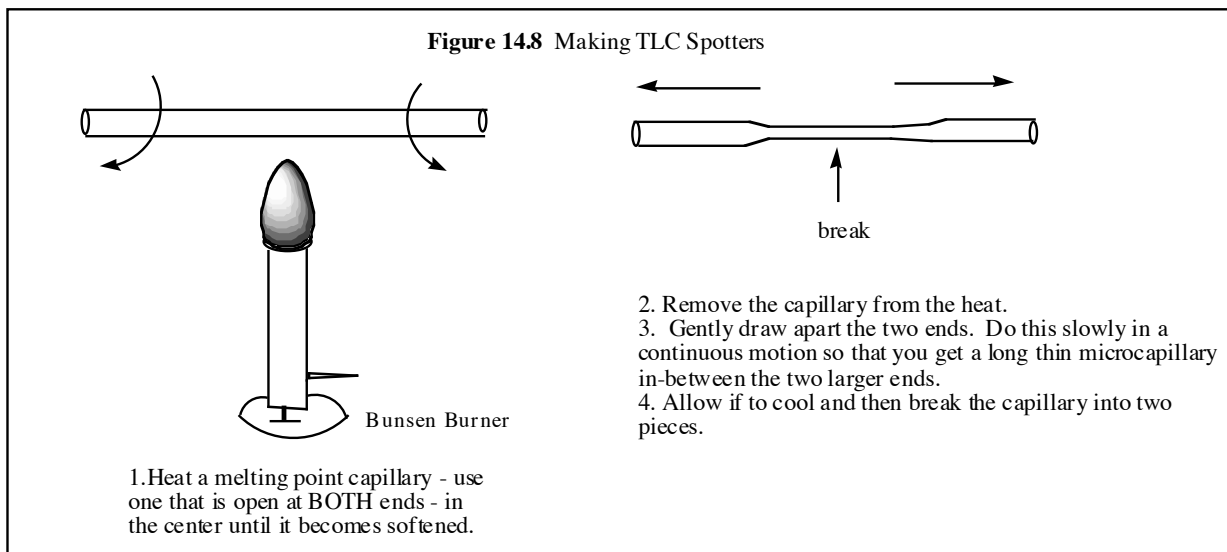


**HANDLE THE TLC plates ONLY BY THE EDGES. THE OIL FROM YOUR FINGERS CAN SHOW UP AS EXTRA SPOTS ON THE PLATES.**

Using the microcapillary, spot a drop of the dye mixture on the baseline of a TLC plate. As discussed in Figure 14.6, draw a light line with pencil one cm from the bottom of the plate. **DO NOT USE INK!** It contains organic compounds and will contaminate the plate. Mark a small dot with your pencil at the point where you are going to apply your compound. In most TLC analyses you can safely put 2 or 3 spots side by side on a single plate. In this case we will put just one spot. You should touch the plate as briefly as possible with the capillary. The idea is to make the spot as small as possible while still having it be visible on the plate. If more material is needed, then allow the first spot to dry and then briefly touch the plate again (re-spot) at the same place on the plate.

Place your TLC plate in the developing chamber as shown in Figure 14.6. Watch as the solvent moves up the TLC plate. When it gets near the top of the plate (it does not have to go all the way up) take it out and quickly draw a line using a pencil at the highest point that the solvent reached. This is called the solvent front

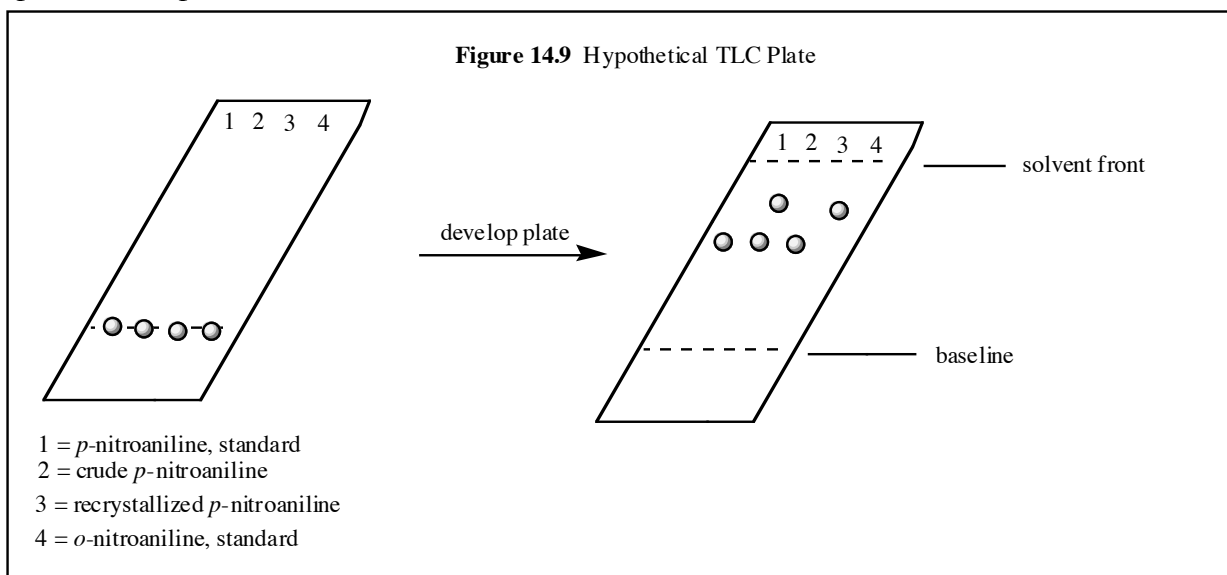
and will be our L value for calculating the  $R_f$ 's. Mark the position of the dye spots with your pencil using a millimeter ruler and find their  $R_f$  values. Record these in your notebook.



### TLC of Nitroaniline

We will now do a TLC analysis of our crude reaction material and our recrystallized material. We will also spot two standards, pure *para*-nitroaniline and pure *ortho*-nitroaniline. The spots will be as follows (see Figure 14.9). Ideally, we should put four spots on a plate. You can try this or you can use one plate for crude material and pure *ortho*-nitroaniline and another plate for recrystallized material and pure *para*.

- Spot 1      pure *p*-nitroaniline (Obtain this from the stockroom.)  
 Spot 2      crude *p*-nitroaniline  
 Spot 3      recrystallized *p*-nitroaniline  
 Spot 4      pure *o*-nitroaniline (Obtain this from the stockroom.)





Make solutions of each of the components by dissolving a spatula tip in 10-15 drops ethanol in a small test tube. Label your test tubes. Develop the plate using methylene chloride as the solvent. Mark the solvent front and the position of each of the spots. If it is difficult to visualize the spots then put the plate in the iodine chamber for a few minutes (The chamber will be provided by the Stockroom.) If the spots are still not visible, you will have to re-do the TLC using a fresh plate. Make the spots darker by using more concentrated solutions and/or make multiple applications (2-3) of the same spot, letting the plate dry each time before re-spotting it at the same place. **But remember to keep the spots as small as possible.**

Sometimes it may be difficult to place all 4 spots on the same plate. If you get the spots too close to the edge of the plate, the spot may migrate off the plate becoming “crooked”. If this happens, you might find it easier to put only 2 spots per plate.

Figure 14.9 gives a **hypothetical result**. Do NOT copy this into your notebook. The  $R_f$  values and relative polarities are simply made-up and not meant to be accurate but what you need to understand from this figure – and from doing your experiment – is how to interpret the developed plate. Was the crude compound a pure substance? If not, what other substance did it contain? If there is another compound present, how can you account for the presence of this compound? Was the overall reaction successful in producing the desired compound? Which isomer of nitroaniline travels faster and why is this?