[MUSIC PLAYING]

PROFESSOR 1: Column chromatography. The purification of chemicals is one of the most important jobs of a research chemist. Perhaps the most common purification technique in an organic laboratory is column chromatography. This technique takes advantage of the different polarities of different compounds to separate mixtures, frequently on the grand scale. Technically, carbon chromatography can be challenging, but with a little practice, you will be able to separate mixtures of compounds consistently and successfully.

As the name suggests, a column of adsorbent, either silica or alumina, is packed into a glass tube with a stopcock at the bottom. The sample is applied to the top of the adsorbent and solvent is run through the column until the compound is flushed out of the bottom. In most cases, flash column chromatography is used, meaning that pressure is applied to the top of the column, resulting in faster running times and better separation.

Before you can run a column, you need to decide on the appropriate conditions. It is vital that you pick a solvent system that will provide good separation. In general, you will use a mixture of two miscible solvents, one polar and one nonpolar.

Luckily, TLC is an effective tool for deciding on the optimal solvent system for your sample. Make a TLC sample by dissolving a small amount of your material in approximately 1 milliliter of solvent. Use this sample to spot several TLC plates, and develop each plate in a different solvent system. Make sure that the adsorbent on your TLC plates is the same as the adsorbent you will use in the column, either silica or alumina.

Most commonly, you will use a solvent system composed of a mixture of ethyl acetate and hexane. But when you are purifying a volatile compound, it is a good idea to use a lower boiling solvent system, such as a mixture of ether and pentane. Mixtures of methanol and dichloromethane are frequently used to purify highly polar compounds. When you are removing small amounts of impurities from a sample, focus on the major constituent.

Once you have found a solvent system that separates the components of your sample, adjust the polarity so that the major, and hopefully the desired constituent, has an Rf of approximately 0.3. If the Rf is too low, then the solvent system is too nonpolar, and it will take a very long time for the material to come off of the column. Alternatively, if the Rf is too high, then the solvent system is too polar, and the material will come off of the column very quickly with poor separation.

When you are separating a mixture of two or more compounds, adjust the solvent polarity such that the midpoint between the spots is at an Rf of approximately 0.3. When you are separating two or more compounds with very different polarity, it will save you time to increase the polarity of the solvent as the column proceeds. Begin the column with a solvent system that puts the least polar compound at an Rf of approximately 0.3. Once that compound has completely come off of the column, slowly increase the polarity of the solvent to a mixture that puts the next spot at an Rf of 0.3.

Continue this until all desired spots have come off of the column. Once you have picked an appropriate solvent system, you need to decide how much adsorbent to use. This video will illustrate the use of a silica gel column, but the same procedure can be followed using alumina.

[WARNING BEEPS]

PROFESSOR 2: Caution. Silica and alumina are highly toxic when inhaled. Handle adsorbents in the hood.

PROFESSOR 1: In cases with good separation, a 20 to 1 mass ratio of silica gel to compound is usually adequate. This means that for 1 gram of compound, you would use 20 grams of silica. It is good to use as little adsorbent as possible, but when the separation is more difficult, ratios of 50 or 100 to 1 may be necessary. Choosing the appropriate amount of adsorbent takes practice. Until you have developed your own intuition, it is a good idea to ask a more experienced chemist for advice.

Weigh the silica into an Erlenmeyer flask in the hood. Use a flask large enough that you don't fill it more than one third full. The last major decision to make is what diameter of column to use.

Different people have very different opinions about this, but in general, it is a good idea to choose a column that will fill 6 to 7 inches with the chosen amount of silica. In a taller column, the different bands of compounds will disperse and overlap. But a shorter column will not have enough surface area to give good separation.

Packing a column with adsorbent can be fairly tricky, and it takes practice to be able to do it well every time. The first step involves plugging the bottom of the column with a small piece of cotton to prevent loss of silica gel. Roll a small piece of cotton between your fingers, and drop it into the column. Tilt and tap the column until the cotton settles into the center depression.

Using a long stick, gently pack the cotton into the hole. Don't pack the cotton too tightly, or it will be difficult to force solvent through the plug. To continue packing the column, you will need an empty beaker, a pipet, a supply of your chosen solvent mixture, a funnel, your pre-weighed silica gel, some sand, and a flash pressure apparatus.

With the cotton in place, clamp the column in a straight vertical position, and use a funnel to pour in a small layer of sand, approximately 1 to 2 millimeters. Now, fill the column with approximately 5 inches of solvent mixture. Make sure that the sand layer is flat and no sand is stuck to the sides. Pour enough of the solvent mixture into the silica gel to form a mobile slurry.

Swirl the silica, and carefully pour it into the column, making sure that you do not disturb the sand layer. Use additional solvent to add the remaining silica. Once you have added all of the silica, rinse the funnel and sides of the column with a small amount of solvent.

Apply pressure to the top of the column and open the stopcock, tapping the sides of the column gently to make sure that the silica layer is flat. Drain the solvent until two or three inches remain above the silica layer. Then close the stopcock and remove the pressure.

[WARNING BEEPS]

PROFESSOR 2: Caution. Make sure there are no bubbles before you add sand.

PROFESSOR 1: Bubbles and inconsistencies in your column will cause serious problems later. To avoid bubbles, use a very wet flurry of silica gel. Gently tap the sides of the column while packing the silica, and apply pressure before opening the stopcock.

When you have achieved a uniform layer of adsorbent, use a pipet to rinse any excess silica down from the sides of the column. Using the funnel, carefully add a small layer of sand. Be careful not to disturb the top of the silica gel. Use a small amount of solvent to wash the excess sand from the sides of the column. Apply pressure, open the stopcock, and drain the solvent to just above the silica gel.

[WARNING BEEPS]

PROFESSOR 2: Caution. Never let the solvent layer drop below the top of the column.

PROFESSOR 1: Now you're ready to load your sample onto the column. There are a couple of ways to do this. If your sample is soluble in the chosen solvent system, then it is easiest to load your sample as a solution. Dissolve your sample in the smallest possible volume of the chosen solvent mixture. If you have trouble getting it to dissolve, try adding a few drops of methylene chloride. It is vital that your sample is completely dissolved before you add it to the column.

Slowly drip the solution of sample around the edges of the column, being careful not to disturb the sand layer. Lower the solvent level to just above the silica. Rinse the flask with a small amount of solvent, and add the rinse in the same fashion. Lower the solvent level again, and repeat the rinse one or two times. It is a good idea to rinse any residual sample from the sides of the column, lowering the solvent level in between each rinse.

Now you're ready to fill the column with solvent. Start by slowly adding solvent with a pipet. Once you have added a nice cushion, slowly pour the remaining solvent into the column, being careful not to disturb the sand or the silica. Done correctly, you should end up with a thin band of sample just below the sand.

When your sample is largely insoluble in the chosen solvent mixture, it is more effective to pre-adsorb the sample onto a small quantity of silica and add it as a dry mixture. To do this, you will need some methylene chloride, a round-bottom flask, and some silica gel. Start by dissolving your sample in methylene chloride and transferring it to the round-bottom flask. Make sure you complete the transfer with a few rinses.

Then add a small amount of silica gel to the flask, and swirl to mix. Before you can remove the solvent on the rotovap, stuff a few chem wipes into the bump trap to prevent contamination of the rotovap with the very fine silica powder. Attach your flask to the bump trap, and concentrate it on the rotovap until you obtain a fine, free-flowing powder. If the mixture remains clumpy or sticky, you may need to re-dissolve the sample and add more silica gel. Don't add it to your column until it is a free-flowing powder.

To dry load your sample, pack the column as before, but leave a small layer of solvent above the sand. Pour the pre-adsorbed silica onto the top of the sand. Use a small amount of the solvent mixture to rinse the flask, and wash the silica down from the sides of the column.

Drain the solvent to the top of the silica, and add a small layer of sand. Carefully fill the column with solvent, as before. One alternative to dry loading is loading your sample as a solution in a solvent that is more polar than the solvent system you're using for your column. You will end up with a thick band of compound and poor separation.

Once your sample is loaded on the column, you must begin running it immediately, and it's a good idea not to take any breaks until it's through. Before you start, make sure you have an adequate supply of your chosen solvent mixture and a nice, big rack of test tubes. Begin running the column by placing a test tube at the bottom of the column, applying pressure to the top, and opening the stopcock.

If the pressure is right, you should have a fairly rapid flow of solvent out of the bottom of the column. You may have to adjust the pressure so that the flow rate isn't a slow drip or an out-of-control stream, but something right in the middle. Don't forget to continually collect the eluant in test tubes. You have to pay attention so that the solvent doesn't overflow.

[WARNING BEEPS]

PROFESSOR 2: Caution. Pay close attention to the solvent level, and refill frequently.

PROFESSOR 1: Now your hard work is over, and it's time to see if it paid off. When your compound is colored, it can be easy to find it in the fractions. But a more reliable method for monitoring your column is with TLC. You can easily spot five or six fractions per TLC plate. Make sure you rinse your spotter in between fractions.

Develop the plates with a solvent mixture that spreads the spots out on the plate. It does not have to be the same solvent system you use for the column. If it goes well, you will end up with a maximum of one compound per fraction, with a few clean factions in between the compounds you're trying to separate.

Unfortunately, it doesn't always work out so well. Overlapping fractions containing more than one compound are the sign of a failed separation. So what went wrong? If all of the material elutes in just a few fractions, then the column may have been too small or the developing solvent too polar. On the other hand, if the compounds are spread out over many fractions, then the column may have been too tall or you used too much solvent to load your sample.

Once the last compound has come off of the column and you have analyzed your TLC plates, it is time to combine the desired fractions. Combine all of the fractions containing the same pure compound in a round-bottom flask. Fractions containing more than one compound should be set aside for further purification. It is a good idea to rinse the test tubes two or three times with a little bit of clean solvent and add those rinses to the flask as well. Don't fill the flask more than half full. Remember, you need to concentrate it on the rotovap. Removing solvent from your compound is the last step in a chromatographic purification.

[WARNING BEEPS]

PROFESSOR 2: Caution. Never discard any fractions until you recover the desired material.

PROFESSOR 1: It is a good idea to clean up the silica before you discard it in the appropriate waste container. When you are sure that there is no more desired material on the column, fill the column with ethanol, place a flask underneath, and push air through the column until the silica is dry and free-flowing. At this point, it will be easy to discard the silica by pouring it into the proper waste container.

When you need to purify a small amount of compound, it is sometimes easier to run a micro-column in a pipet. On small scale, the packing and sample loading steps are greatly simplified. It is easiest to pack a micro-column completely dry without any solvent.

Plug the end of the pipet with a small piece of cotton. Then add a couple of inches of adsorbent and a thin layer of sand. Next, add your sample, pre-adsorbed onto a small amount of adsorbent. Top it off with a thin layer of sand, and you're ready to go.

To run the column, place a collection flask or a test tube underneath the pipet, and use another pipet to add solvent to the top of the column. Keep adding solvent until your compound has come off of the column. Make sure you don't let the column run dry.

This video has outlined the basic steps that you will use to perform a purification using column chromatography. Now it's time to get in the lab and try it for yourself. Remember, this video is intended to help you prepare for lab by providing a demonstration of the proper experimental technique. It is not intended as a replacement for reading your lab manual or the supplementary material.

In order to become a great experimentalist, it is important that you understand both theory and technique. Now it's your turn. Good luck.

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