Column Chromatography: Separation Through Polarity

Imagine a chemist has a clear nonvolatile homogeneous solution of a solid and a liquid they want to separate. The chemist can't separate the solution through distillation because there is a solid in the mixture, and they can't separate the solution through recrystallization because there is a liquid in the mixture. Recall that one can only preform distillation if they are trying to purify/separate a mixture of liquids, and one can only perform recrystallization of they are trying to purify/separate a mixture of solids. How might a chemist go about separating this mixture?

Thus far we haven't covered a technique used to purify the type of solution the chemist is faced with. But what if there was a simple technique the chemist could use? Is it possible a person can take a mixture of chemicals and separate them, regardless of whether the chemicals are a solid or a liquid? The answer is yes, by using column chromatography.

Column Chromatography and Its Phases

Column chromatography is technique used to separate and purify mixtures of solids and/or liquids. Column chromatography is based on polarity. Chemicals can be separated in a mixture if they have different polarities.

There are two different phases used in column chromatography, the stationary phase and the mobile phase. The stationary phase (usually silica gel) is polar, and the mobile phase is nonpolar. Because the two phases differ in polarity, chemicals can be separated based on which phase they have stronger intermolecular interactions with.

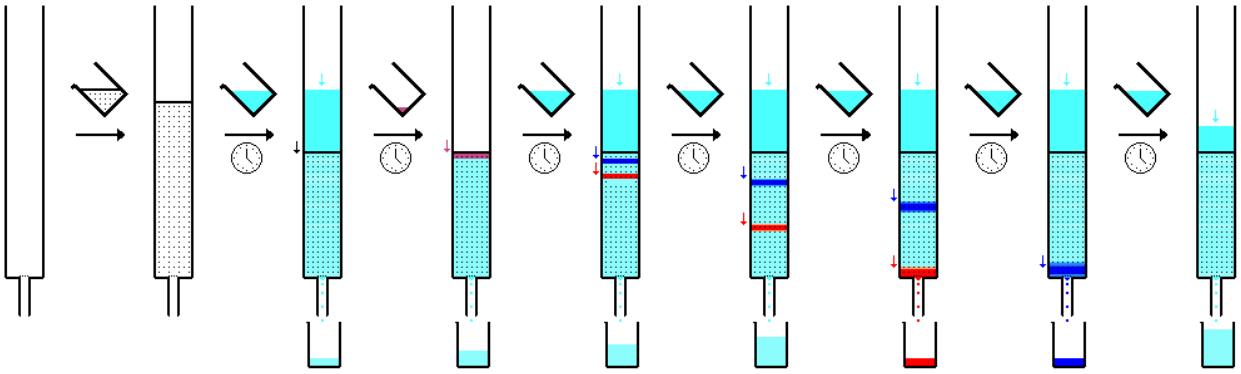

Figure 1. An example of an experiment utilizing column chromatography.[1]

For example, nonpolar molecules will travel with the nonpolar mobile phase and exit the column rather quickly because they have an affinity (attraction) for each other. On the other hand, a polar molecule will travel much slower through the column because it has an affinity for the polar stationary phase. An easy way to see this is in Figure 1.

Figure 1 is an example of an experiment where column chromatography was utilized. In this figure, the second column has a white section with dots throughout in the column which represents the polar stationary phase. The blue liquid that is constantly being poured into the column is the nonpolar mobile phase. The sample is purple, and once it's loaded (put on the top of the column) and allowed to run through the column, the two chemicals start to separate. In this picture, there is a blue chemical and a red chemical. In this case, the red chemical is more nonpolar, as its traveling through the column faster and elutes first. Moreover, the blue chemical is more polar because it has a higher affinity to the polar stationary phase and elutes slower than the other chemical.

The Importance of Thin Layer Chromatography (TLC)

Being able to run multiple TLC plates while doing column chromatography is a must, because it lets the chemist know when each chemical is going to elute from the column. Similar to the column, the stationary phase on a TLC plate is polar silica and the mobile phase, which the chemist chooses, is nonpolar. Therefore, the more polar chemical will be closer to the origin of the TLC plate because the polar molecule has stronger interactions with the polar chemical. On the other hand, the nonpolar chemical will travel with the nonpolar mobile phase up the TLC plate towards the solvent front because they have a high affinity for each other. Figure 2 is in reference to the same sample used in figure 1. The purple sample is on the origin of the TLC plate. Based on how far the two chemicals travel, the red chemical is more nonpolar than the blue chemical because the red chemical is closer to the solvent front while the blue chemical is closer to the origin.

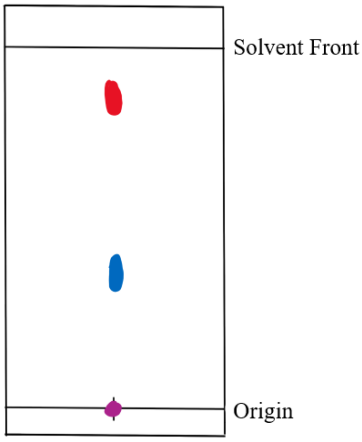

Figure 2. A TLC plate showing proper separation of the sample.

It is important to note that when doing TLC for column chromatography, a chemist usually tests multiple fractions on one TLC plate in order to save time and materials

Factors that Effect Separation

The purpose of column chromatography is to separate the chemicals within a sample. As mentioned previously, chemicals separate based on polarity. In addition to having the correct polarity for the sample, there are many different things that contribute to achieving good separation.

COLUMN PACKING

Having a nicely packed column is the first step in ensuring sufficient separation is possible. A poorly packed column is evident by the stationary phase having cracks or bubbles, as shown in Figure 3 where the white curved lines indicate the cracks running through the silica gel. These cracks lead to poor separation because they allow the samples to run through the column at different rates. For example, imagine there was a crack in the column shown in figure 1. If this was true, then all of the red chemical would not come out at the same rate. Instead, some of the red chemical could still be mixed with the blue chemical, or the original red band could split into two.

Figure 3. A cracked, poorly packed column.[2]

Moreover, it is very important that the column never runs dry. A column runs dry when there is no mobile phase running through the column. A dry column leads to cracks and/or bubbles appearing in the column, which leads to poor separation as previously mentioned.

Table 1. Common Polar and Nonpolar solvents.

Polar Solvents	Nonpolar Solvents
Methane	Hexanes
Ethane	Diethyl ether (ether)
Dimethyl sulfoxide (DMSO)	Toluene
Dichloromethane	Benzene

MOBILE PHASE

Having the correct mobile phase is arguably the most important aspect when trying to achieve optimal separation. Recall that the mobile phase is nonpolar and the stationary phase is polar. In order to successfully separate chemicals within the sample, it is imperative the mobile phase has a stronger affinity for one chemical over the other. Usually a mobile phase is a mixture of two or more solvents. Moreover, the mobile phase that is used in the column is typically the same

solvent that is used when developing the TLC plate of the fractions. For reference, Table 1 indicates common polar and nonpolar solvents.

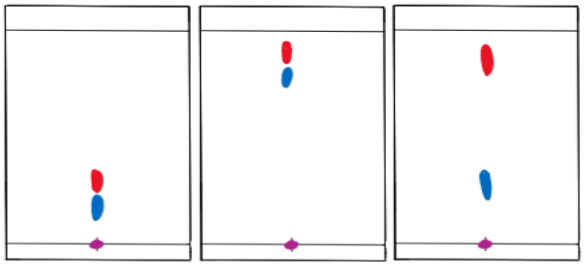

Figure 4. Three TLC plates showing the effect of mobile phase on the separation of chemicals.

In order to determine the correct mobile phase to use, it is customary to test multiple mobile phases on the sample using TLC before starting the column. Examine the three TLC plates to the right in Figure 4. In this example, as all the others, the red chemical is more nonpolar than the blue chemical. The first TLC plate on the left is taken from a single fraction where the mobile phase is not sufficient. The red and blue chemicals are not separated effectively, and they are too close to the origin. This means that the mobile phase is too polar. In order to correct this, a more nonpolar solvent, such as hexanes, should be added to the mobile phase to lead to better separation. The second TLC plate in the middle is an example of when the mobile phase is too nonpolar. This is evident by the fact that both chemicals are not separated and are close to the solvent front. In order to correct this, a more polar solvent, such as ethanol, should be added. In the third and final TLC plate on the right, there is ample separation between both chemicals meaning the mobile phase is sufficient and will lead to proper separation.

Let's dive a bit further and think about a circumstance where a chemist has three chemicals in one sample, as shown on the TLC plate in Figure 5. There is good separation between the blue chemical and the other chemicals on the plate. However, the separation between the red and green chemicals is not the best. The chemist has tried everything they could think of, and the separation shown on the TLC plate in figure 5 is the best they could do. How is the chemist supposed to separate all three chemicals effectively? The trick is to gradually change the polarity of the mobile phase when doing the column. Gradually adding a more polar solvent to the mobile phase will increase the polarity of the mobile phase, meaning the green chemical will have stronger interactions with the polar stationary phase. Because the green chemical has a stronger interaction with the stationary phase it will travel slower down the column than the red chemical. Therefore, the red chemical will elute first, then the green, and lastly the blue chemical.

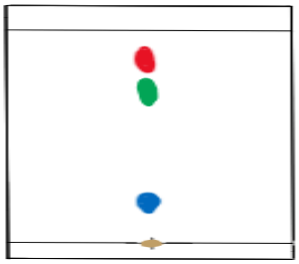

Figure 5. A TLC plate of a solution containing three chemicals, each with a different polarity.

FRACTION SIZE

A fraction is simply what the chemist collects from the column as it elutes. There is a plethora of fraction sizes, ranging from as small as 10 mL to large as 200 mL. The size of the fraction depends on how large the column is itself and how good the separation of the sample is. For example, if the sample has two chemicals that have similar Rf values, a smaller fraction size should be utilized. On the other hand, if two chemicals have a drastically large difference in their Rf values, then a larger fraction size could be utilized. Recall the definition of retention factor, or Rf, is the distance the solute travels divided by the distance the solvent travels.

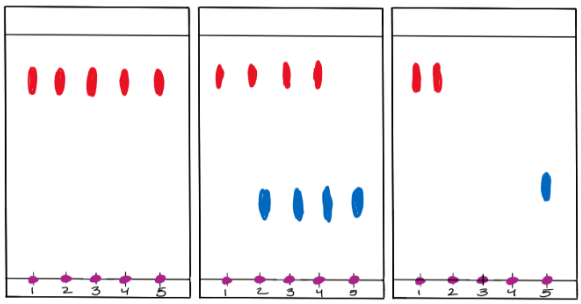

Figure 6. Three TLC plates showing the effect of fraction size on the separation of chemicals.

It may not seem like it, but setting a standard fraction size is immensely important when considering not only good separation, but also time. Take a look at the three TLC plates in Figure 6.

The first TLC plate on the left is an example of when the fraction size is too small. As evident in this TLC plate, multiple fractions are necessary for the red chemical to elute. It's important to note that it can take multiple

fractions for a chemical to elute, but if it takes over 12 fractions for one chemical to elute, the fraction size is too small.
The second TLC plate in the middle shows a plate where the fraction size is too big. In fraction 2 through 4, both chemicals are in each fraction. That means the chemist has to run those fractions through the column again, because the chemicals didn't separate. When the fraction size is too big, there is a waste of chemicals because the chemist has to use more mobile phase, and a waste of time because the chemist has to put the fractions with both chemicals through the column again.
The third TLC plate on the right shows a TLC where the fraction is the correct size. When the correct fraction size is chosen, it should be clear when each chemical elutes. In this case, the red chemical elutes in fractions 1 and 2, and the blue chemical is starting to elute in fraction 5. Moreover, given a sufficient fraction size there should be a few fractions where no chemical present, as in fraction 3 and 4.

Conclusion

When doing an experiment utilizing column chromatography, many factors are involved in order to successfully complete the experiment and separate chemicals within the sample. First, the column must be properly packed to ensure each chemical runs through the column at the same rate. Next, the correct mobile phase needs to be determined using TLC to confirm the chemicals will separate properly when running the column. The last factor that should be taken into consideration when doing a column chromatography experiment is what size fraction is needed. If the wrong fraction size is used, the experiment might have to be redone, so choosing the correct fraction size is pivotal.

Bibliography

1. *Column Chromatography Sequence*; 2008.
2. *I did my first silica column in a few years... I think it went terrifically wrong*; 2019.