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PROFESSOR: Thin Layer Chromatography, or TLC, is a common analytical technique used in organic laboratories. TLC can be helpful when you are trying to identify the components of a mixture or assess the purity of a sample. It is also used to monitor both reactions and purifications. It can take some time to become a pro at TLC, but this video should help to familiarize you with the basic technique.

TLC plates are composed of a thin layer of a polar adsorbent, either silica or alumina, which has been bound to a plate of solid support, generally plastic or glass. In most cases, a fluorescent powder is also mixed in with the adsorbent to aid with visualization, as you will see later in this video.

A sample is applied to a TLC plate using a thin glass spotter. Micropipets, such as the one shown here, can be used and are conveniently commercially available. However, in many laboratories, TLC spotters are prepared by heating and pulling capillary tubes or disposable Pasteur pipets. One benefit of making your own spotters is that these homemade spotters are usually thinner than commercially available micropipets. You can watch the advanced thin layer chromatography video for a demonstration of this procedure.

Once a TLC plate has been spotted with a sample, it is developed in a developing chamber that can easily be assembled from a glass jar with a lid, a piece of filter paper, and 5 to 10 milliliters of an appropriate developing solvent. Before you get started, make sure you also have a pair of tweezers, a pencil, and a ruler close at hand.

Assembly in the developing chamber is very straightforward. First, slide the filter paper into the jar so that it is flat against the wall. Next, pour in approximately 8 millimeter layer of the appropriate developing solvent into the jar. Tilt the jar to moisten the filter paper, and close the lid to prevent evaporation.

You should end up with a layer of solvent no more than 5 to 8 millimeters deep. The moist filter paper ensures that the air in the chamber is saturated with solvent vapor. It prevents evaporation of solvent from the TLC plate during development. Once you have put together your developing chamber, it is time to prepare the TLC plate.

In this video, plastic-backed alumina plates will be used. For more information on preparing glass plates, you can watch the advanced TLC video. Before you start, there are a few things to remember.

Always use a pencil and never a pen to mark your TLC plates. Ink is soluble in organic solvents and will be developed along with your sample. Make sure that you always mark and spot your sample on the dull and not the shiny side of the TLC plate. Remember, the dull side is coated with the adsorbent. Even though you should be wearing gloves while handling TLC plates, it is important that you do not touch the face of the plate with your fingers. Oils from your skin or other contaminants can adsorb to the plate and affect your results.

Before you can apply your sample to a TLC plate, it is important to mark the plate so that you can keep track of where the sample is applied. One way to do this is to draw a straight line approximately 1 centimeter from the bottom of the plate. Draw small ticks through the line at each point where you will apply a spot of sample. It is important that the spots are not too close to the edge of the plate, or evaporation from the sides of the plate will result in inconsistent results. It is also important that the spots are not too close together, or you will end up with overlapping spots after you develop the plate.

Applying your sample to the plate is probably the trickiest part of TLC, and it may take some practice before you can consistently apply the right amount of sample in a small enough spot. Your TLC sample should be fairly dilute, containing approximately 1% to 2% of the desired compound. If the sample is too dilute, you will not be able to visualize the spots. If the sample is too concentrated, you will observe large streaky spots on your final plate.

To spot the plate, dip the glass spotter into the sample and touch it lightly and quickly to the plate. Wait briefly for the solvent to dissolve before spotting again. Generally, 1 to 3 spots will suffice.

It is crucial that you do not leave the spotter on the plate for too long, or you will end up with large diffuse spots. It's difficult to separate mixtures on a TLC plate when the spots are too big. Try to keep the spots 1 to 2 millimeters in diameter. The smaller, the better.

Once you have applied your sample and the spots have dried, it's time to develop the plate. Using your tweezers, pick up the plate and place it in the developing chamber. Make sure that the solvent level is below the spots on the plate, otherwise you will end up with your sample dissolved in the developing solvent. Replace the cap to prevent evaporation of solvent off of the plate, and try not to let the edges of the plate touch the filter paper. This will disturb the capillary motion of the solvent on the plate.

Keep a close eye on the solvent front. Do not let the solvent get closer than 5 to 10 millimeters from the top of the plate. When the solvent front gets too close to the top, evaporation from the top of the plate becomes a problem. The spots keep moving up the plate, but the solvent front appears to stop. This leads to incorrect Rf values.

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When the solvent front has reached an appropriate height, remove the plate and immediately draw a line at the solvent front. This line will be necessary for the calculation of Rf values. When the compound of interest is brightly colored, no extra steps need to be taken to visualize the spots. However, most organic compounds are colorless and cannot be seen on a TLC plate with the naked eye. Fortunately, most TLC plates contain an additive that causes the plates to fluoresce under ultraviolet light.

Certain UV active compounds are capable of quenching this fluorescence. As a result, they appear as dark spots on the glowing TLC plate. UV lamps such as this one are commonly found in laboratories for just this purpose.

Just place your plate under the lamp, turn it on, and mark the spot with a pencil. A number of TLC stains have also been developed to aid in the visualization of spots that cannot be seen under a UV light. You can watch the advanced thin layer chromatography video for a demonstration of a few of these staining procedures.

TLC data is described in terms of Rf values. Under a specific set of conditions, a particular compound should always exhibit the same Rf. Let's quickly run through the procedure for calculating an Rf value.

First, measure the distance from where the spot started on the plate to where it ended up. Always measure from the center of the final spot. We'll call this distance A. Next, measure the distance from where the spot started to where the solvent front ended up. We'll call this distance B.

Do not measure from the bottom of the plate. This is a common mistake that will lead to incorrect Rf values. The Rf value is defined as the ratio between the distance the spot moved, A, and the distance the solvent moved, B. This value is dependent on the polarity of the compound and the polarity of the developing solvent. Polar compounds will have lower Rf values than nonpolar compounds under the same developing conditions.

This series of plates illustrates the effect of solvent polarity on Rf. As the polarity of the developing solvent is increased from left to right, the spot moves further up the plate. The value of A gets larger while the solvent front, B, stays the same. Increasing the polarity of the developing solvent generally increases the Rf values of all of the spots.

It is a good idea to use a developing solvent that gives you Rf values between 0.2 and 0.8. This will generally give you the most effective separation when you have more than one compound in your sample. For example, a mixture of two compounds was spotted and developed in hexanes, a nonpolar solvent. The final plate shows only one spot with a very low Rf. The same mixture was developed in ethyl acetate, a polar solvent, and once again, the final plate shows only one spot, this time with a very high Rf.

When a mixture of hexane and ethyl acetate was used as a developing solvent, the two spots were resolved. Notice that both spots are near the center of the plate. TLC is frequently used to compare the identity of two compounds.

In a perfect world, TLC data would be consistent from plate to plate and chamber to chamber. Unfortunately, small deviations in development conditions affect the observed Rf values. As a result, it is most convincing to compare Rf values of different samples on the same TLC plate.

For example, you know that the unknown sample marked with a question mark consists of either compound A, compound B, or a mixture of the two. You can use TLC to determine the identity of the unknown. You will need two TLC plates.

Make three marks on the first TLC plate, one for the unknown, one for A, and one in the center for the co-spot. Do the same with the second plate, except substitute B for A. On the first plate, spot compound A on the center mark and one of the side marks, and allow both spots to dry well. Drain the excess liquid from the spotter onto a paper towel. And rinse your spotter in clean solvent before switching to a different sample.

Now, spot the unknown mixture on the center mark and the other side mark of the first plate. Repeat this procedure with compound B on the second plate, and develop both plates. Now, compare the two plates. You can see that the co-spot with A shows two distinct spots while the co-spot with B shows only one. These results indicate that the unknown sample contains only compound B.

In this video, we have surveyed the basic techniques that you need to know to use thin layer chromatography in the lab. You have learned how to set up a developing chamber, how to mark and apply your sample to a TLC plate, how to develop the plate in your developing chamber, how to visualize the spots on your developed plate using a UV lamp, and how to correctly calculate Rf values. You have also learned how to choose an appropriate developing solvent for your system and how to compare the identity of two compounds.

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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