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PROFESSOR: TLC Advanced. While the basic TLC video covers the essential steps for using thing layer chromatography, a few advanced techniques may also come in handy. For instance, learning how to cut your own glass TLC plates or pull your own spotters is essential in a research lab environment. It is also important to know how to visualize spots using TLC stains as well as UV light. This video will cover the advanced techniques involved in carrying out thin layer chromatography.

In the basic TLC video, you learned how to set up a developing chamber, mark and spot a TLC plate, develop the plate, visualize the spots using a UV lamp, and calculate Rf values. You also learned how to choose a developing solvent and compare the identity of two compounds using a co-spot. This video will demonstrate some advanced techniques commonly used in research laboratories. You will learn how to cut glass TLC plates from large plates, how to pull spotters from capillary tubes, and how to stain TLC plates to visualize spots.

Let's start out with the TLC plates. Plastic-backed TLC plates such as those used in the basic video are found in many laboratories because they are cheap, usually pre-cut into a usable size, and if not, easy to cut with scissors. In spite of these conveniences, many chemists prefer glass-backed TLC plates. They can withstand more heat than a plastic-backed plate, which is important when you are using stains to visualize spots. They also frequently offer better resolution.

Unlike plastic plates, glass plates are generally purchased as large squares, approximately 20 centimeters by 20 centimeters. This means that it's up to you to cut the plate into smaller plates of usable size. With a few well-placed cuts, you can generally get approximately 40 to 60 small plates from one big plate. The small plates are commonly 5 centimeters long and about 1 to 2 centimeters wide.

Cutting glass TLC plates without breaking them is something of an art. It will take some practice, but here are a few tips to get you started. First, you will need a glass cutter like the one shown here to score the glass and allow you to break it in a straight line. Start by making three well-placed cuts approximately 5 centimeters apart. This will give four long strips that can be cut into individual plates.

Start by placing the large glass plate adsorbent side down on a clean, dry surface. Use a pencil and a ruler to mark the cuts that you are going to make. Next, use the glass cutter to score a line in the plate.

You may be tempted to score the plate repeatedly to deepen the cut in the glass, but this will actually make it more difficult to break the plate in a clean, straight line. Instead, firmly score the glass with one sweep of the glass cutter, and using the edge of your bench for support, break the plate in a clean, straight line. Continue cutting the long strip into individual TLC plates until you have amassed a nice stockpile.

With your plates ready to go, now it's time to pull some TLC spotters. While commercially available capillary spotters can be used to apply samples to TLC plates, many people prefer to pull their own spotters. This is because pulled spotters have a smaller diameter, so you can apply smaller spots to the plate. This means that you get better resolution, and you are able to spot more spots per plate. Because you can conserve plates, this makes pulling your own spotters the economical choice.

Put the center of a capillary tube into the blue flame from a Bunsen burner, and hold it there until the center starts to soften. Then remove the capillary from the flame and pull on the ends of the tube. This causes the center of the tube to stretch and narrow. This will take a little bit of practice. If the tube isn't hot enough when you pull it, you won't be able to stretch it, but if you leave it in the flame for too long, it'll turn into a saggy mess.

Once you have heated and pulled the capillary tube, break it into two pieces at the center. You should end up with two fine-tipped TLC spotters from every capillary tube. With a bit more experience and an acetylene torch, you can also pull TLC spotters from Pasteur pipets. On a good day, you can get dozens of TLC spotters from one pipet. But for safety reasons, this technique is best learned from an experienced lab mate.

With your plates and spotters in hand, you're ready to learn about using some TLC stains. A standard TLC station in an organic laboratory consists of a UV lamp, which you learned about in the basic video, a variety of TLC stains, some paper towels, and a heat source, either a heat gun or a hot plate. Before you do any staining, spot and develop your plate as usual and allow all of the solvent to evaporate from the surface. Then dip your plate into the stain. Allow excess stain to drain back into the jar, and dry the back of the plate on a paper towel.

Don't be discouraged if you don't see any spots on your plate just yet. You have to heat the plate to activate the stain. Turn on the heat gun, and hold the plate just above the barrel.

Watch closely, and you should see your spots appear. Don't heat for too long or you'll end up with a blackened TLC plate. While numerous TLC stations have been developed, some good common ones are Phosphomolybdic Acid, PMA, Ceric Ammonium Molybdate, CAM, and potassium permanganate.

Let's finish up with a few general tips for using TLC stains. Remember that staining is a destructive form of visualization. The stain will react irreversibly with the compounds on your plate. This means that you should always view your plate under the UV lamp prior to staining. Not all compounds will show up in all stains, so you may have to screen a variety of stains to find one that works for you.

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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