CHROMATOGRAPHY

Zakarian Group, 2008

I. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is probably the most important analytic technique available to you. It is inexpensive, and very rapid. It allows analysis within ~5 min directly from your reaction mixtures.

TLC should be performed very accurately. Do not be satisfied with sloppy TLCs.

In your analysis of a reaction, if you observe a spot at the baseline (Rf 0.0), make every effort to find conditions where this spot is seen at Rf at least 0.2 or more by increasing solvent polarity. If you see a spot at the front (Rf 1.0), make every effort to record an additional TLC where this spot is seen at Rf at least 0.8 or lower by decreasing solvent polarity.

Thus, in many cases I will expect to see several high-quality TLCs for any given new reaction.

TLC PIX

A. **Plates** (available within group)

Silica Gel:

analytical

EMD 5715-7
20x20 cm, 0.25 mm, glass

Aluminum oxide

analytical
EMD-5713-7, 20x20 cm, 0.25 mm, glass

B. **TLC plate sizes**.

The following sizes only are used in the lab:

4x1.5 cm: for each new reaction, three tracks. The first track is the starting material, the third track is a reaction mixture, and the middle track is a mixed spot (starting material plus the reaction mixture). For new reactions, you starting point is ALWAYS a TLC where the starting material appears at Rf of about 0.5. It is critically important to adhere to this technique, and for each new reaction I will

expect to see a TLC following this standard.

4x1 cm: for familiar compounds and reactions, one or two tracks

4x4 cm: for chromatography.

In order to maintain uniformity throughout the group, no other sizes are permitted.

C. **Detection of spots**.

There is no universal visualizing agent. Careful TLC work requires the use of several techniques.

In general, the following order is followed for each new TLC analysis

First) U.V. Lamp. Compounds containing a chromophore are visible. You should have a basic idea what kinds of chromophores are present in your starting material or expected products. MARK OBSERVED SPOTS WITH A PENCIL.

Non-destructive. Placing under UV Lamp again will show the spots.

Second) Iodine Vapor. Place you plate in a closed TLC chamber. Within a few minutes reddish-brown spots will appear. Take you TLC plate out and circle the spots, they will soon disappear. This color is presumably the result of solution of iodine in the organic compounds.

The technique is usually non-distructive and is more general than method 1.

Third) Charring. Dip a SOLVENT-FREE, DRY plate in charring solution for 1-3 seconds, allow the excess reagent to drain off. Thoroughly clean the back of the plate, then heat it on a heating plate until you see spots. Do not over heat. Remove the plate, let cool, then clean the back of the plate thoroughly again.

It is important to dip dry, solvent-free TLC plate in order to get an acceptable quality result without smudged spots. Drying can be generally achieved by gentle heating and blowing off the solvent off the TLC plate prior to dipping.

Do not be satisfied with sub-standard results. Keep running the TLC plates until you get a solid result, until you can make a reliable conclusion about what is going on in your reaction. For each TLC analysis you perform, think about exactly what you are trying to determine. In most cases, this means you will need to obtain high-quality TLC plates for the same new reaction under SEVERAL DIFFERENT CONDITIONS.

II. COLUMN CHROMATOGRAPHY

A. Sorbents available in the group.

B. Flash Chromatography Procedure.

Choosing Conditions. The first step in successful chromatography is to develop a proper solvent system. This is measured by the separation achieved on our standard precoated TLC plates. Optimum results will occur when a) maximum separation is achieved and b) the desired compounds have an Rf value of ~0.3.

TLC can be used to divide separations into two categories. Simple separations have Rf differences of > 0.2 while difficult separations are <0.2. For simple separations loading of the sample can be higher, and solvent polarity is usual. For difficult separations, decrease sample loading, and/or use somewhat lower than usual polarity of eluent

Packing the columns. Different techniques can be used. Always pack columns to no more than 6 inches of silica to minimize interactions with the glass wall of the column.

Guidelines for Sample Loading. Samples are loaded by dissolving you mixture in a MINIMUM amount of your eluent solvent and placing the solution on the top sand layer with care using a pipette, and then press solvent down to the silica level. This is followed by at least five rinses.

If your mixture is poorly soluble in the eluent, the following procedures can be used.

A) Same as above, but use a MINIMUM amount of dichloromethane. The proceed to run your column with the desired eluent.

B) Dissolve your compound in at least 10 mL of dichloromethane and add 1 to 5 times its weight in silica. Carefully concentrate to dryness on the rotovap then pump for a few minutes on a vac line. Using a spatula, scrape the silica on to a sheet of paper and carefully add it onto prepared column. Tap to pack and distribute the silica evenly. Add 1/2 inch of sand.

Standard loading:

up to 50 mg	13 mm column

50 to 300 mg	20 mm column
300 mg to 1 g	25 mm column
1 to 5 g	50 mm column
more than 5 g	75 mm column

Fraction Collection. For columns up to 25 mm we use 13 mm test tubes. The amount of each fractions changes for different column sizes

13 mm	up to a third
20 mm	half
25 mm	full

Obviously, difficult separations require smaller fractions than usual.

For larger columns 25 mm test tubes or Erlenmeyer flasks are used.

Armen Zakarian, June 2008

Santa Barbara, June 2008