

## Thin Layer Chromatography (TLC)

You will be using prepared tlc plates which are research quality (\$1.50/sheet). Each large TLC sheet will be carefully cut to provide mini tlc plates. Do not touch the adsorbent with your fingers, hold the plates at the sides. Do not use ink on the plates, write in pencil *lightly*.

For a developing chamber, you will use your 4 oz. wide-mouth jar with a piece of filter paper *ca.* 3 inches in diameter (trim if necessary). The half TLC plate you receive can be cut width-wise into thirds to make three mini-tlc plates about 4 cm wide. Cut evenly and carefully using sharp scissors. These mini tlc plates will fit into the 4oz. jars. 2.5-3 mL of ethyl acetate should be used as the developing solvent to obtain the proper depth with a baseline 0.5 cm above the bottom of the mini tlc plate. The solvent front should be allowed to run as close to the top of the plate as possible but not to the top, stop at least 1 mm from the top. These plates take about 5 min. to develop.

1. Prepare at least 5 micropipettes for sample spotting.
2. Prepare samples of your aspirin, acetaminophen, and caffeine by dissolving *ca.* 10 mg of compound in 1.0 mL of 1:1 methylene chloride/ethanol in a stoppered vial.
3. Practice your spotting technique on a sheet of filter paper (or towelling) until you are satisfied that you can apply narrow diameter samples and that your pipettes are reliable.
4. Draw a very light **pencil** line at 0.5 cm from the bottom of your mini tlc plate (it is very important that the pencil does not gouge out the adsorbent).
5. Spot samples on the line in a predetermined order or, even better, code the samples in pencil at the top of the tlc plate. Include a sample of the unknown you have chosen.
6. Observe your spotted samples under ultraviolet light to see if they are in appropriate positions on the starting line of the tlc plate.
7. Carefully place the tlc plate into the developing chamber so that the bottom of the plate is flat in the ethyl acetate developing solvent and the plate does not touch the filter paper. Close the developing chamber carefully without disturbing your tlc plate. The solvent front should be rising along the tlc plate evenly.
8. When the solvent front is 0.5 cm from the top, remove the tlc plate from the developing tank and immediately mark the solvent front on the plate with a pencil. Let the tlc plate dry in the hood then observe it under UV light. Circle the spots with a pencil. Record the R<sub>f</sub> values.
9. Visualize the tlc plate in an iodine chamber. Record any differences you observe.

The number of variables affecting absolute chromatographic values is large. To the obvious variables of solvent, medium, and sample, can be added:

1. activation of adsorbent; 2. amount of sample applied; 3. solvent composition; 4. amount of adsorbent; 5. solvent developing distance; 6. chamber saturation (actually an error if not saturated, not a variable); 7. impurities in both phases; 8. temperature; 9. room humidity; 10. distance of the sample spot from the chamber solvent level;...

With so many variables, qualitative information is usually obtained by separating known solutions (standards) on the same sheet as the sample.

