PRACTICAL #3:
THIN LAYER CHROMATOGRAPHY

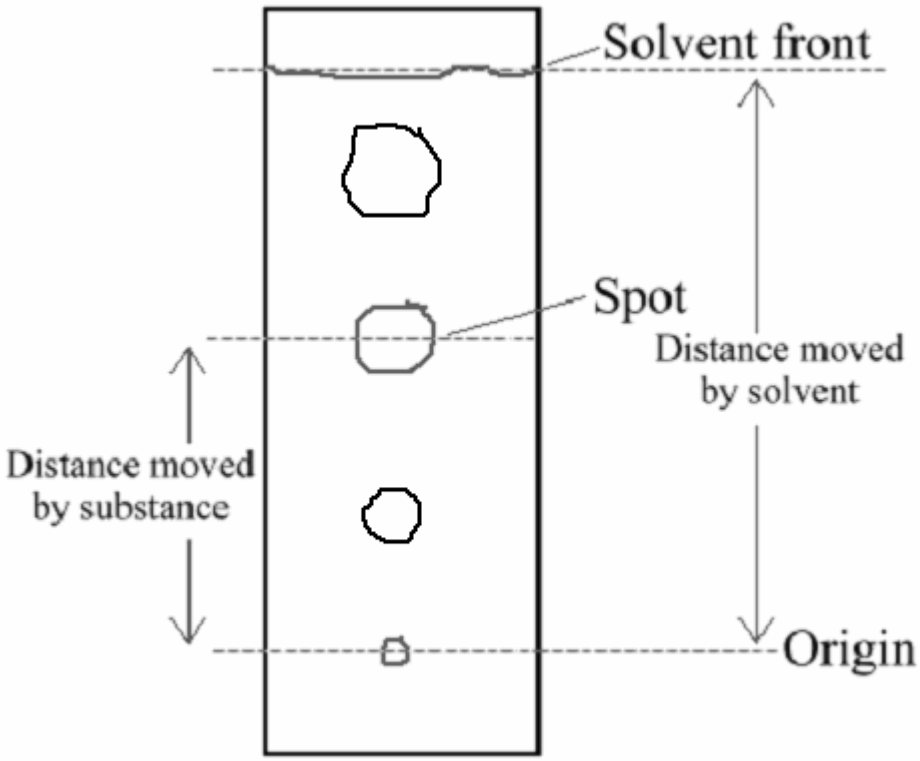
Thin Layer Chromatography (TLC) is a technique used to separate the components of organic mixtures and to identify organic compounds. It involves applying a minute amount of a solution of the sample to be analyzed to a thin layer of a solid adsorbent (usually silica gel or aluminium oxide) as a spot. The spot may contain between 1 and 200μg of the solute, which may be a complex mixture.

The solid adsorbent is spread as a thin layer on a glass plate or sheet of aluminium foil or plastic. This layer of adsorbent constitutes the **stationary phase**. The plate is then placed in a container containing a solvent, called the **mobile phase**, which travels up the adsorbent by capillary action. The substances in the spot have varying affinities for the adsorbent and the mobile phase described by a *partition coefficient*, K. The higher the value of K, the higher the concentration of the substance in the mobile phase relative to its concentration in the stationary phase. Since there is a dynamic equilibrium, with continual exchange between the adsorbed material in the stationary phase, and dissolved material in the mobile phase, movement of the mobile phase "pulls" the components of the sample along with it, but at different rates. Substances with a small value of K move more slowly and substances with a large value of K move more rapidly. The solvent forming the mobile phase is sometimes called the *eluant*. The separation of the components as they move up the plate is called *development* of the chromatogram. It results in a series of spots distributed up the chromatography plate. If they are coloured they may be visible, but most compounds are colourless, and so have to rendered visible. For this a reagent may be sprayed on the plate to form a coloured substance, or the plate may be viewed under ultraviolet light.

A chromatogram is recorded by its R_f (retention factor) value which is given by:

$$R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent}}.$$

as shown in the diagram below:



An important factor in determining the value of the partition coefficient for a particular substance is its dipole

moment, its polarity. More polar solvents tend to attract more polar solutes, whilst less polar solvents are better at dissolving less polar solutes. For example, hexane, which is non-polar, dissolves non-polar substances such as waxes, and makes a good mobile phase for their separation using chromatography. The substances in this practical are more polar, and require a more polar solvent for their separation.

In this practical you will attempt to separate a mixture of benzoic acid, benzanilide and acetanilide. You may have to find a suitable solvent, or a mixture of solvents, or one may be given to you. As well as the mixture you will subject pure samples of the substances to the same procedure so that you can identify the components of the mixture.

Pre-Lab Assignment

1) a) Draw the structures of benzoic acid (phenylmethanoic acid), *N*-phenylbenzamide (benzanilide) and *N*-phenylethanamide (acetanilide), as well as those of the compounds in the table below, and give the dipole moment of each substance. These are possible components of a mixture which you will separate.

   b) Which of the three substances is

      i) the most polar?

      ii) the least polar

2) Complete the table for the eluting solvents given:

Solvent	***Dipole moment/D***
Methanol	
Propanone (acetone)	
Hexane	
Ethanenitrile (acetonitrile)	
Ethyl ethanoate (ethyl acetate)	
Methylcyclohexane	
Ethanol	
Methylbenzene (toluene)	
Dichloromethane	

Method

You will be provided with two small glass jars with covers to be used as the chromatography tanks.

You will also be provided with commercially prepared chromatography plates made of aluminium sheet. Cut a rectangular piece about 1 cm wide and almost the height of the jar. Using a pencil, make a straight line approximately 1 cm from the bottom of the narrow end of the plate- this line is called the **origin**.

Add mobile phase to the chromatography tanks until it is about 0.5 cm deep, close them and allow them to become saturated with the solvent vapour. This will prevent the solvent from evaporating as it rises up the plate.

Dissolve a tiny quantity of the sample in approximately 1 cm^3 of ethyl acetate in a dimple on a white ceramic tile. Spot a few microlitres of the sample on to the origin line on the plate, using a micropipette. Dry with an electric blower. A small spot obtained from multiple applications is better than a larger spot obtained from fewer applications. Too much sample in your spot is probably the greatest danger.

Repeat with pure samples of the components, making 4 plates in all.

Put the spotted plate into the jars, two at a time. The origin must be **above** the level of the mobile phase. Allow the solvent to travel until the solvent front is approximately 0.5 cm from the top edge of the plate. Do not disturb the jar until the development is complete. When the solvent front reaches the desired level, carefully

remove the plates and make a horizontal mark with a pencil to indicate the solvent front in each case. Dry the plates with the blower.

Visualize the chromatograms by viewing them under an ultraviolet lamp. The adsorbent contains a fluorescent indicator which reveals the spots under ultra violet light. Draw a pencil line around each spot. A pure substance will give one spot. A mixture will give more than one spot as each component should give a separate spot if separation is complete. Calculate the R_f for each spot.

Based on your observations, identify the components of the mixture on your chromatograms. Suggest which component has the highest affinity for the mobile phase.