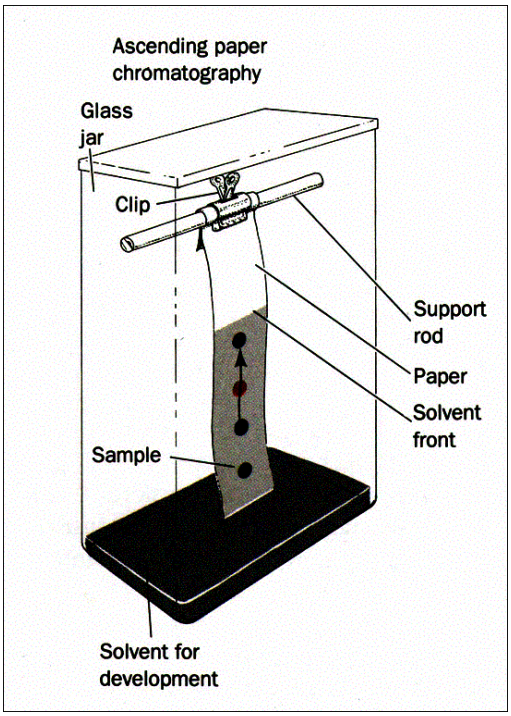
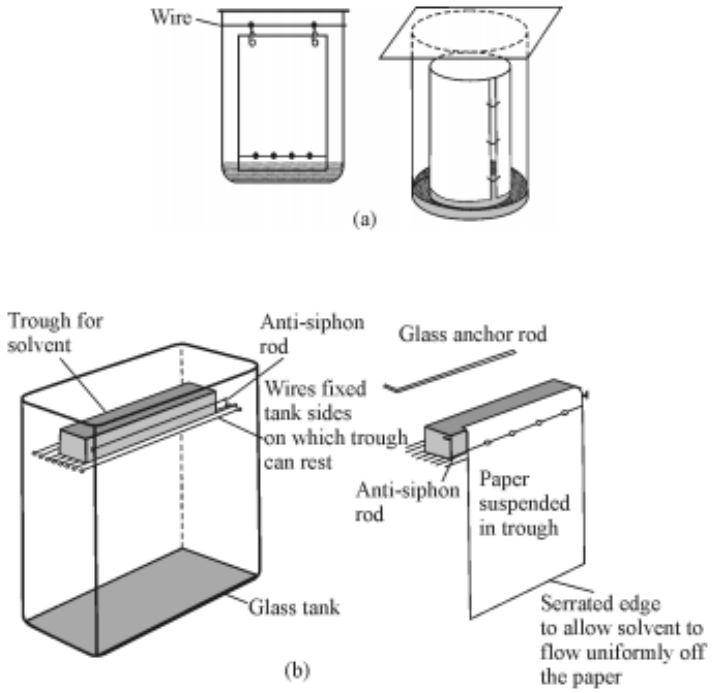
PAPER AND THIN LAYER CHROMATOGRAPHY

Chromatography is a technique used to separate the components of a mixture. There are two common types of chromatography – adsorption and partition chromatography. Adsorption is the binding of a substance to the surface of another substance due to weak intermolecular forces. In partition chromatography, a substance may be subjected to partition between two liquids but the system is never allowed to reach the steady state because of the continued influx of fresh liquid. In every variant of the chromatography technique, a mobile phase (often termed eluent) passes over or percolates through a stationary phase. The mobile phase is either a moving liquid or a flowing gas and the components of the mixture to be separated are initially expose to it and thus travel along with the flow. The stationary phase is a solid (in the case of adsorption chromatography) or is a liquid adsorbed on the surface of a solid (in the case of partition chromatography). In the latter case, the solid which 'holds' the attached liquid is often referred to as the 'support'. It is finely divided to maximise surface area of contact between the mobile phase and stationary phase.

PAPER CHROMATOGRAPHY:





Paper chromatography is an example of partition chromatography, the 'other' liquid being water which is adsorbed strongly by hydrogen bonding on the cellulose fibres in the paper. The technique of paper chromatography consists of a sheet of cellulose filter paper which serves as a stationary phase or separation medium. A small amount (usually a few micrograms) of solute is placed in a small area near the end of strip. A solvent is allowed to move from the end of the paper by capillary action and after equilibration for some fixed period, the solute migrates from its initial point of application. The components of mixture are separated completely or partially in distinct coloured zones or are located by the application of different reagents or by applying ultra-violet fluorescence.

R_f value: It is a characteristic parameter called retardation factor (or retention factor) and abbreviated as R_f. It represents the position of an ion or a substance with respect to solvent phase. R_f of a solute is defined as the ratio of the rate of movement of the solute to the rate of movement of the solvent. R_f is most commonly used in paper and thin layer chromatography and is considered as a characteristic of nature of the solute sample which may, of course, change with the solvent phase.

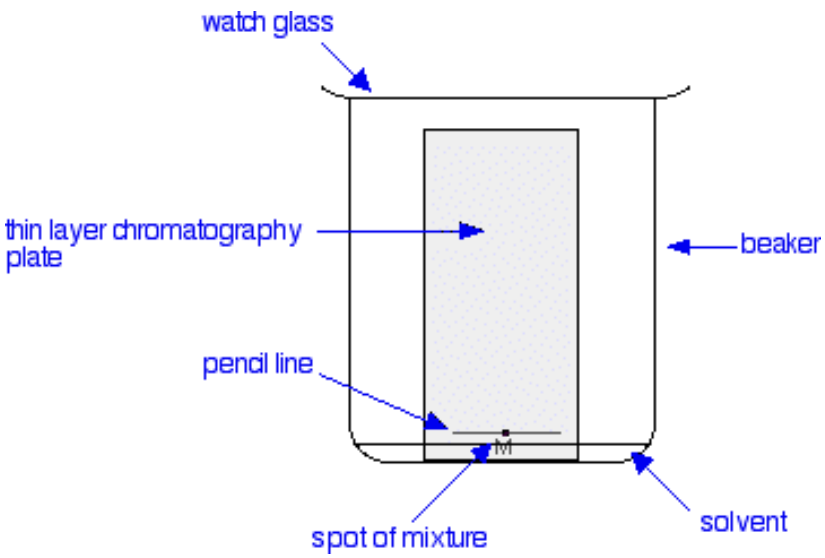
It describes relative migration of the solute with respect to the solvent and may be represented as:

$$R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$$

In order to have good separation, two spots must not overlap with each other and these must be symmetric without any 'tailing'. In order to avoid tailing, different solvent mixtures, mixed in proper ratio, must be tried. It has been observed that R_f values are influenced by the impurities in the paper and solvent, temperature and saturation of the atmosphere. R_f values are reproducible under identical conditions which includes the solvent (eluent) used and the grade and type of chromatography paper.

The advantage of paper chromatography is that the completed chromatograms are easily stored for future study. However the disadvantage lies in the fact that the components tend to spread and stream (or tail) as the solvent proceeds to its final level. The problem of streaking arises due to the cellulose in the paper. The technique of paper chromatography is primarily used for qualitative identification though it could also be used for quantitative determination but with a poor precision.

THIN LAYER CHROMATOGRAPHY:



Thin layer chromatography (TLC) is a form of adsorption chromatography used mainly to identify organic compounds or to ascertain whether or not they contain impurities. It is a powerful qualitative analysis. The technique offers better resolution than paper chromatography and is commonly used in the drug industry for purification due to its reliability. The stationary support plates (usually made of glass or tough plastic) are coated with a slurry of adsorbent (alumina, silica gel, cellulose powder are most common), which usually has had a binder such as starch or calcium sulphate added to help it adhere to the backing material. Care is taken to ensure that the layer deposited is of an even thickness. The choice of the mobile phase is largely empirical, but often are organic mixtures that optimise the solubility of the components in the sample.

The following precautions must be followed while handling TLC plates:

1. The surface of the TLC plates should not be touched. The plates should be handled carefully by holding at the edges so as to avoid any contamination due to sweat.

2. The plates should be cleaned thoroughly so as to remove any extraneous material that might contaminate the adsorbent.

The detection of the spots in TLC is easier than in paper chromatography as silica and alumina used as support are inert and hence strongly reactive reagents can be used to locate the compounds. If the components of the mixture are colourless, their positions must be revealed by spraying the completed, dried plate with an appropriate agent. A universal technique involves the use of iodine vapours for colourless or non-fluorescent spots. It consists of exposing the developed plate to iodine vapours which interact with the sample components, either chemically or by solubility to produce colour. Alternatively some other colour forming reagents called chromogenic reagents may be used.

**Note: distance measurements are taken from the marked origin or starting line. Pencil is used for any reference markings for subsequent measurements since graphite is insoluble and will not interfere with the results produced by the sample.