C 241

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QUALITATIVE ANAYSIS OF MIXTURES USING TLC (CHROMATOGRAPHIC TECHNIQUE)

AIM

To analyze a sample mixture qualitatively using TLC, a chromatographic technique.

THEORY

TLC i.e., **Thin Layer Chromatography, a technique** which is used to analyze a small spot of the sample is made at one end of a glass or plastic plate that has been coated with a thin layer of silica gel. In a process known as "development" the plate is then immersed spot-end down in a pool of solvent (the exact solvent used depends on the sample and is determined by experimentation). The solvent is allowed to move up the plate by capillary action (the silica gel "soaks it up"). Compounds present in the sample are carried up the plate by the solvent. However, different compounds generally move at different rates. Therefore, if the sample is a mixture of compounds it will separate into a series of spots at varying distances up the plate (fig 1). If the sample is pure (i.e., only a single compound is present) then only one spot will result.

If the compounds in the sample are colorless then the spots will be hard to see against the white back ground of the silica gel and a process for "visualizing" them must be used. A UV light source can be used for this purpose if the silica gel contains a small amount of a fluorescent substance. (The commercial TLC plates used in this experiment have silica gel to which the compound, fluorescein, has been added). Under UV light the spots will show up as dark spots against a bright background.
How far a particular compound moves from the original spot depends on the rate at which the solvent moves it. The fastest the solvent can move the compound is the same rate at which the solvent moves. In this case the compound forms a spot at the same distance from the original spot as the distance the solvent was allowed to move (the spot's Rf value = 1.0, see below). At the other extreme, a compound may be moved so slowly by the solvent that its spots remains where the original spot was placed (Rf = 0.0, see below). By measuring the distance of spot (solute) and solvent moves, we can quantify the rate of migration of any compound using the ratio referred to as the $\mathbf{R_f}$ value.

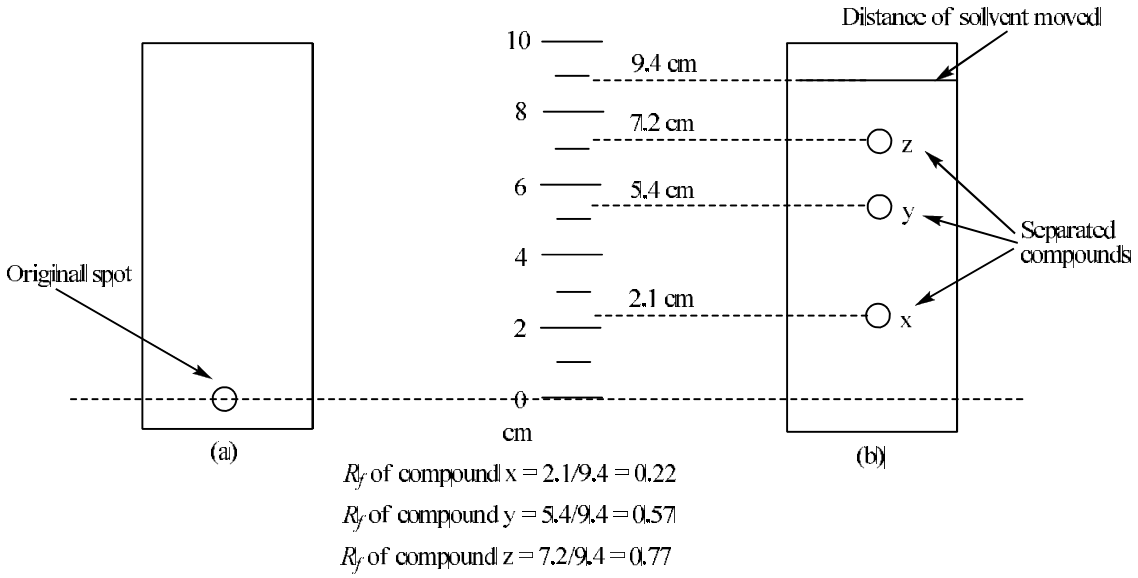
$$R_f = \frac{Distance\ moved\ by\ solute\ front}{Distance\ moved\ by\ solvent\ front}$$

The $\boldsymbol{R_f}$ value of a particular compound is an identifying characteristic of the compound just like its boiling point and melting point. Therefore, spots with identical $\boldsymbol{R_f}$ values in two different samples can reasonably be concluded to indicate the presence of the same compound in both samples. For example, if TLC analysis of an unknown sample gave two spots with $\boldsymbol{R_f}$ values of 0.22, 0.57 and 0.77 (as in fig 1[b]) then the sample can be concluded to consist of a mixture of three compounds. Further, if TLC analysis of a known sample of pure caffeine resulted in a spot with a $\boldsymbol{R_f}$ value of 0.23, then one may reasonably conclude that one of the compounds present i n the unknown sample is caffeine. (The small difference between the two values, 0.22 and 0.23, is ascribed to unavoidable random error in the measurement of $\boldsymbol{R_f}$ values, which at best are accurate to no more than ±0.02).

MATERIALS REQUIRED

TLC plates, diethyl ether, hexane, ethanol, dichloromethane, chloroform, o- & p- nitroaniline, turmeric powder, TLC chamber, ruler, capillary tube, spatula, forceps etc.

Example illustration



PROCEDURE

1. Take approximately 25 mg of the sample in a small labeled test tube and add 1.0 mL developing solvent to it.
2. Mix and stir vigorously to dissolve the solid.
3. Now obtain a TLC plate. Draw a light pencil line at the straight edge about 1 cm from one end of the plate.
   NOTE: The plate should be handled using forceps so as to avoid contamination.
4. Use a capillary micropipette to make a small spot of the solution (made in step 1) on the plate.
5. Place the spot at the midway point along the pencil line you drew.
6. Develop the TLC plate by placing it in a beaker that has been filled with developing solvent to a level of less than 1 cm high (the spot on the TLC plate should be above the level of the solvent).
7. Cover the beaker with aluminum foil immediately after the TLC plate is immersed.
8. Allow the solvent to migrate up the TLC plate until it is about one centimeter from the top. Do not allow the solvent line to reach the top of the plate.
9. Remove the TLC plate and mark the level to which the solvent rose with a pencil. Allow the solvent to evaporate off the plate in the hood and then visualize the plate. Outline all spots with a pencil.
10. Measure the distance the solvent moved as well as the distances of all spots. Carefully sketch the TLC plate in the space provided on the report sheet.

Results:

TABLE, OBSERVATIONS AND CALCULATIONS

S.No.	Distance moved	Solvent	Compound	Spots in sample 1	Spots in sample 2

Sketch of developed TLC plate

Comment on the results.