PART V

THIN-LAYER CHROMATOGRAPHY

5. Thin-layer chromatographic studies of coumarins :

All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type which is in equilibrium with a stationary phase.

The 'thin-layer chromatography' has the technical simplicity of paper chromatography and the speed of gas chromatography. This is also some times called as "thin film", "open column", "chromatostrip" or "chromatoplate" chromatography. In essence, this is a type of adsorption chromatography where the adsorbent is a thin layer of some solid deposited on a glass plate support. In operation it is analogous to paper chromatography. The applications of the thin-layer chromatography mushroomed after 1958.

The use of "TLC" is now almost routine in natural product, pharmaceutical and lipid laboratories. The small amount of material involved and the speed with which results are obtained make thin-layer chromatography a valuable tool for preliminary exploration of many kinds. It can be used for the study of a system or situation, for the study of a single reaction and for the isolation of reaction products.

Thin-layer chromatography can predict the solvent and adsorbent required for the column chromatography for the removal of small amount of contaminants in a sample. Thin-layer chromatography can be used to determine characteristic component-patterns for drugs, plant extract and biochemical preparations.

The most widely used adsorbends in thin-layer

chromatography are : silica gel, alumina, kieselguhr and cellulose.

Silica gel, is by far, the most extensively used adsorbent in thin-layer chromatography. The thin-layers of adsorbent used for thin-layer chromatography are supported and held in place by glass plates. The glass plates used may be flat and smooth. The size and thickness of the glass plates depend on the mechanical device used to prepare the layers. It is essential that the glass plate be as clean as possible.

The Stahl type of spreading device is characterised by a moving slurry reservoir and stationary plates. The plates are arranged in a continuous surface on a support having a raised edge on two sides to hold the plates in position during the coating operation. The reservoir, machined in such a way that it produces the desired layer thickness, is then passed over the plates.

After the slurry is spread on a plate, it should be allowed to stand for at least 30 minutes and then dried in an oven.

The solution of the sample (to be tested) is prepared by dissolving the sample in a suitable solvent. The application of the samples to bound layers is carried out by touching the tip of a filled capillary, micropipette, or microburette to the adsorbent layer in a manner analogous to that used in paper chromatography.

In ascending chromatography, most thin-layer

chromatograms are developed by placing a glass plate, containing the sample on a bound, thin layer, in a verticle position in a closed, saturated system such that the bottom of the layer dips into the developing solvent. After the solvent ascends 10 to 15 cms., the plate is removed, dried and visualized. The visualization of developed thin-layer chromatogram can be carried out in many ways. The method used in a specific case will depend upon the type of compounds to be visualized, the nature of adsorbent and the purpose of chromatogram.

The fluorescent compound or the compounds containing conjugated double bonds show up as coloured spots when viewed in an ultraviolet light.

The following compounds were applied on thin-layer chromatography : (1) 4-hydroxycoumarin, (2) 4,7-dihydroxy-coumarin, (3) 4-hydroxy-6-methylcoumarin and (4) 5,7-dihydroxy-4-methylcoumarin.

Procedure :

The thin layers were made of Silica Gel G. and were prepared as per standard technique using Shandon Model spreading device. The thickness of the layer was $\sim$ 4 mm.. The coumarins were dissolved in acetone-chloroform (97:3) mixture and the same solvent was used as the developer.

2-3 mg. of the compound dissolved in 1/2 ml. solvent and 2-3 drops were placed on TLC plate with the help of a capillary. The plate was developed for one hour and dried in atmosphere and observed under ultraviolet light.

Observations :

The TLC plates of 4-OH, 4-OH-6-Me and 4,7-diOH showed two distinct spots, very well resolved. On placing the plates in iodine vapour for some time two clear brown spots developed. Whereas 5,7-diOH-4-Me did not show any resolution.

The two bands of 4-hydroxycoumarin were cut out of the plate and the compound was eluted out with acetone. After evaporating the acetone, the obtained compound was dissolved in methanol and qualitative ultraviolet absorption spectra was taken. Both the fractions gave identical spectral pattern, which was identical to the original compound 4-hydroxycoumarin.

Comclusion :

The above observation supports the earlier conclusion that 4-hydroxycoumarins are resolved into two components by chromatograph.