General Information

Principles

Thin Layer Chromatography has long been over 100 years one of the most useful forms of chromatography. In TLC, an aliquot of sample solution is spotted on a porous layer of adsorbent material. This sample mixture is then resolved into its individual components by their differential migration as they are carried through the adsorbent by the developing solvent. The developing solvent wicks through the adsorbent by capillary action alone; no external source of pressure is required.

The best TLC separation media are produced by spreading a concentrated slurry of adsorbent particles as a layer of uniform thickness on the flat surface of an inert backing material (usually glass, but aluminum and plastic are also used). Careful evaporation of the slurry solvent produces a highly uniform adsorbent layer. In addition to providing an inert support for preparing the thin adsorbent layer, the backing material also contributes strength for ease of handling.

In principle, a wide variety of adsorbent materials can be used for the production of TLC layers. In practice, the versatility of silica gel has led to its acceptance as the adsorbent of choice for the great majority of TLC separations. The steps in performing TLC are simple, yet can be considered fields of study by themselves. They are - sample preparation, sample application, chromatographic development, and evaluation of the chromatogram.

Sample Preparation

This step involves, in the words of Dr. Joseph Touchstone, "favorably increasing the analyte/'junk' ratio." Although the most important issue here may appear to be cleaning up your sample, it also may involve physically crushing a sample. Other processes that may occur in this step are sampling, extraction steps, and filtration or concentration of components of interest Thorough preparation of samples is an important prerequisite to a successful TLC separation.

Sample Application

The objective of the chromatographic separation will typically specify how the sample should be applied.The most common method is by way of a glass capillary. With a capillary the sample can be applied as either a circular spot or series of smaller adjacent spots creating a band. A much longer band referred to as a streak is also commonly used but only on preparative thickness TLC plates. It is always beneficial to keep the sample spot or band as small as possible or resolution will be adversely affected. A special TLC plate option that can help with all manual sample application but especially with large volumes of very dilute samples is the concentration or preadsorbent zone.

Chromatographic Development

The most commonly used separation technique is by way of a TLC plate standing vertically in a glass developing chamber. This is known as ascending chromatography. A separation may also be performed in a specially designed horizontal chamber. Solvent flowing across the plate is still achieved by capillary action with this method, however, gravity does not slow the speed of the separation as much. Another specialized method of separation is 2-dimensional development. With this a single spot is applied near a corner of the plates. After chromatography in the first direction, the plate is dried, rotated 90° and developed in the second dimension.with another mobile phase.

Evaluation of the Chromatogram

Evaluation can be as simple as a visual inspection under ultra violet light to determine the existence of a component. Or it could be as complicated as exposing the plate to a chemical spray, followed by heat charring and densitometric scanning to compare the sample to standards on the plate and achieve an accurate measurement of how much of a component is on the plate. Anyway you look at evaluation, the one constant is the calculation of the retention factor (Rf value). This value is measured as the relative distance traveled from the spot origin to the mobile phase solvent front. As long as all other variables are kept constant, the Rf value of a sample component should remain constant and be reproducible.

Inherent Advantages of TLC

TLC permits the simultaneous analysis of many samples in the same time period required for one HPLC analysis. Samples and standards are analyzed under exactly the same conditions rather than serially as in HPLC. TLC uses a fresh, new adsorbent for each analysis. This insures reproducible results and eliminates the HPLC problems of adsorbent contamination from previous analyses and lost efficiency from worn columns. Also, with the use of smaller developing tanks, the amount of developing solvent required is much less than with HPLC. TLC does not require complex, costly maintained instrumentation. The investment for performing successful TLC can be ten to one hundred times less than for HPLC. TLC simplifies methods development. Unlike chromatography performed on columns, the sample components remain in the adsorbent and can always be located and retrieved for further experimentation.

Applications of TLC

TLC is generally used for one of three purposes.

1) QUALITATIVE Analysis : To determine the presence or absence of a particular substance in a mixture. A rough estimation of the level of the substance may also be performed.

2) QUANTITATIVE Analysis : To determine, precisely and accurately, the amount of a particular substance in a sample mix.

3) PREPARATIVE Analysis : To purify and isolate a particular substance by separating it from any contaminants.

All three cases share the common procedures of sample application, chromatographic separation, and sample component visualization. The conditions under which these procedures are performed vary according to the required end result. Some typical requirements and conditions are shown below.

Factor	Qualitative	Quantitative	Preparative
Sample Volume	2-50µL	0.1-0.5µL	50-1000µL
Sample Amount	10-200g	50-500ng	5-500mg
Sample Appl.Precision	±10%	±1%	NA
Layer Thickness	250µm	150µm	500-2000µm
Development Distance	12cm	5cm	15cm
Development Time	15-30min	4-8min	25-60min
Visualization Type	Visual	Visual & Densitometric	Visual