

Video Article

Performing 1D Thin Layer Chromatography

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Abstract

Thin Layer Chromatography (TLC) is a chromatographic method used to separate mixtures of non-volatile compounds. A TLC plate consists of a thin layer of adsorbent material (the stationary phase) fixed to an appropriate solid support such as plastic, aluminum, or glass¹. The sample(s) and reference compound(s) are dissolved in an appropriate solvent and applied near the bottom edge of the TLC plate in small spots. The TLC plate is developed by immersing the bottom edge in the developing solvent consisting of an appropriate mobile phase. The mobile phase is allowed to move up the adsorbent layer by capillary action. As the solvent moves up the TLC plate, it carries with it the components of each spot and separates them based on their physical interactions with the mobile and stationary phase.

Video Link

The video component of this article can be found at <http://www.jove.com/video/5499/>

Introduction

Chromatography involves the separation of a mixture of compounds by distributing the components between two phases². The stationary phase is fixed in place while the mobile phase is allowed to flow through the stationary phase, carrying the components of the mixture with it. The properties of compounds such as solubility in the mobile phase and the strength of interaction with the stationary phase affect the rate which they are carried through the medium. Since different types of compounds have different physical properties, they are carried at different rates allowing for separation of the components of a mixture.

This video will demonstrate the separation and visualization of mixtures of compounds using the technique of one-dimensional thin layer chromatography.

Protocol

1. TLC Plates

1. Common adsorbents for TLC are silica gel, alumina, and cellulose. TLC plates are commercially available with a variety of properties. Choose a TLC plate and cut it down to an appropriate size (approximately 5 cm x 5 cm is sufficient for most applications). For glass backed TLC plates, score the glass using a ruler and a glass cutter and carefully break along the line.

2. Spotting

1. Dissolve the sample in a suitable solvent to make an approximately 1% solution. If possible the solvent should be nonpolar. Column chromatography fractions and other dilute solutions may be used without dilution, if the solute is present at a concentration between 0.2% and 2.0%.
2. Mark the baseline with a pencil about 1.0 cm from the bottom of the plate. Position the spots at least 1.0 cm from the edge of the plate and label them appropriately.
3. Spots may be applied using a glass capillary. To spot the TLC, dip the capillary into the solution to draw in a small quantity of liquid. Gently touch the tip to the desired location on the TLC plate and remove it immediately.
4. Alternatively, apply spots with a microliter syringe by delivering approximately 1 μ l of solution for each application.
5. Spots may be applied successively at each location, taking care not to disturb the surface of the adsorbent with the spotter. Allow solvent to dry in between applications.

3. Choosing a Developing Solvent

1. It is preferable to use the least polar solvent possible for good separation. Common solvents for TLC include hexane, ethyl acetate, dichloromethane, and methanol (**Table 1**).

2. A convenient way to find an appropriate mobile phase is to spot the TLC plate with a sample. Apply enough solvent directly to the spot to form a circle of solvent 1-2 cm in diameter. Mark the circumference of the circle. Upon visualization an appropriate solvent will show well-separated rings, with the outermost ring about 50% of the distance from the center to the solvent front.
3. It may be necessary to adjust the polarity of the mobile phase by choosing two miscible solvents and testing them in varying proportions. Examples of common mixtures are hexanes with ethyl acetate and dichloromethane with methanol.

4. Development

1. Place the spotted TLC plate in a developing chamber containing the appropriate developing solvent. The solvent line should be below the baseline marked on the TLC plate.
2. The developing chamber can be a jar with a lid, or a beaker covered with aluminum foil or plastic film. Use the smallest container available that will accommodate the TLC plate.
3. Do not allow the solvent to reach the top edge of the plate. When the solvent front - the boundary where the wet part of the adsorbent ends - is 5-10 mm from the top the plate, remove the TLC plate from the developing chamber and mark the solvent front with a pencil before the solvent dries.

5. Visualization

1. Colored spots may be visualized immediately and marked with a pencil. Often, spots are not visible and therefore must be visualized by some other method.
2. Often, the TLC adsorbent contains a fluorescent indicator. Spots may be visualized using a hand-held ultraviolet lamp. Compounds that quench fluorescence will appear as dark spots when the plate is irradiated with short-wave (254 nm) UV light. Fluorescent compounds will produce bright spots when irradiated with UV light of an appropriate wavelength. Mark the center of each spot with a pencil.
3. Spots may also be visualized by applying a visualizing reagent or stain to the TLC plate. The visualizing reagent may be applied by dipping the plate into the reagent, or wiping the plate with a cotton ball saturated with a noncorrosive reagent.
4. A 20% solution of phosphomolybdic acid in ethanol is useful for the visualization of most organic compounds. The spots appear upon heating of the plate with a heat gun or in an oven.
5. Other reagents may be used for visualization of specific classes of compounds. For example, ninhydrin reagent is used for amino acid visualization and 2,4-dinitrophenylhydrazine for aldehydes and ketones.

6. Analysis

1. The R_f value is the ratio of the distance a compound travels up a TLC plate to the distance the solvent travels. This may be determined by measuring the distance from the baseline to the center of the spot and dividing the value by the distance from the baseline to the solvent front.

$$R_f = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent}}$$

2. The R_f value of a compound is characteristic of its physical properties and dependent on factors such as thickness and activity of the adsorbent, temperature, and sample size.
3. To confirm that an unknown is identical to a known compound, a standard should be spotted on the same TLC plate. Identical substances will have the same characteristic R_f value.

Representative Results

An example of a typical TLC plate is shown in **Figure 1**. An unknown compound 'A' may be compared to known standards 'B' through 'E'. Determination of the R_f value for each component is achieved by spotting of each respective compound, development of the TLC, and visualization. The value of the R_f of unknown compound 'A' is calculated by measuring the spot height, y , and dividing by the solvent height, z . Comparison of this value to the R_f determined for each of the standards allows for the identification of the unknown compound.

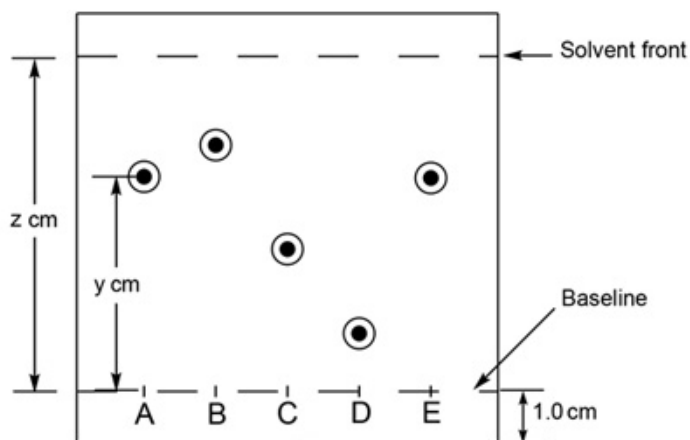


Figure 1. Schematic of a TLC plate. The retardation factor should be consistent between experiments as long as the conditions are held constant.

Eluotropic Series for Silica Gel


Hexane		Increasing eluting power
Petroleum ether		
Pentane		
Dichloromethane		
Chloroform		
Diethyl ether		
Ethyl acetate		
Ethanol		
Water		
Acetic acid		
Methanol		

Table 1. Eluotropic Series for Silica Gel. A list of common mobile phases in order of increasing eluting power.

Discussion

TLC has a number of practical applications in the laboratory. TLC may be used to identify unknown compounds and unknown components of mixtures *via* comparison with standards. TLC is commonly used to monitor the course of a chemical reaction and assess the purity of the product through the comparison of relative amounts of reactants, products, and by-products on successive chromatograms over time. TLC can also be used to determine if a substance purified by recrystallization, distillation, or another method still contains a significant amount of impurity.

When similar compounds cannot be resolved with TLC, they can be further separated based on another physical property, known as a two-dimensional separation. In one example, TLC was used to separate broad lipid classes (triglycerides, sterols, fatty acids, etc.) secreted by mammalian sebaceous glands. The different classes were then further separated by mass spectrometry³.

In microbiology, TLC is used in bioautography screenings for novel plant-based antimicrobial compounds⁴. Once compounds have been separated from an extract of the plant in question, the bands can be applied directly to microbial cultures. Bands that are observed inhibiting growth are then further studied as likely candidates.

TLC is a straightforward method to determine an appropriate mobile phase for separation of a mixture by column chromatography. Additionally, it is used to determine the composition of the various fractions collected during a column chromatography separation, so that fractions containing the desired compound can be identified and collected.

Disclosures

No conflicts of interest declared.

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