

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a quick, sensitive, and inexpensive technique used to determine the number of components in a mixture, verify the identity and purity of a compound, monitor the progress of a reaction, determine the solvent composition for preparative separations, and analyze the fractions obtained from column chromatography. This unit is mainly aimed at novice experimenters, describing in detail the strategies and principal steps for performing a TLC experiment, with illustrations of the relevant instruments, as well as approaches for obtaining and understanding results

OVERVIEW AND PRINCIPLES

The first widespread application of partition chromatography on a planar surface was paper chromatography, introduced in the 1940s. However, paper chromatography was gradually replaced by thin-layer chromatography (TLC), which has become one of the most routinely used chromatography techniques (Ettre and Kalasz, 2001). TLC is also a liquid-solid adsorption technique where the mobile phase ascends the thin layer of stationary phase coated onto a backing support plate resembles column chromatography (UNIT 6.2), where the solvents (eluent) flow down through the column's adsorbent. However, unlike column chromatography, TLC is a quick, sensitive, and inexpensive technique that only requires a few micrograms of sample for one successful analysis. TLC is commonly used to (1) determine the number of components in a mixture; (2) verify the identity and purity of a compound; (3) monitor the progress of a reaction; (4) determine the solvent composition for preparative separations; and (5) analyze the fractions obtained from column chromatography. Like all forms of chromatography, TLC involves a dynamic and rapid equilibrium of molecules between the two phases (mobile phase and stationary phase). However, TLC differs from all other chromatographic techniques in the fact that a gas phase is present, which can influence the results of separation significantly. Between the components of the mobile phase and its vapor, an equilibrium will be established gradually (also called chamber saturation). The part of the stationary layer that is already wetted with mobile phase also contributes to the formation of the equilibrium (Fig. 6.3.1). During development, molecules are continuously moving back and forth between the free and adsorbed states (Fig. 6.3.2A). A balance of intermolecular forces determines the position of equilibrium and thus the ability of the solvent to move the solute up the plate (also see Strategic Planning for details). This balance depends on (1) the polarity of the TLC coating material, (2) the polarity of the development solvent, and (3) the polarity of the sample molecule(s). For example, with a sample consisting of two compounds A and B as illustrated in Fig 6.3.2B, if the molecules A spend more time in the mobile phase, they will be carried through the stationary phase more rapidly and move further in a certain time. While molecules B are adsorbed to the stationary phase more than A, B molecules spend less time in the mobile phase and therefore move through the stationary phase more slowly, and do not move as far in the same amount of time. The consequence is that A is gradually separated from B as the mobile phase flows (ascends).

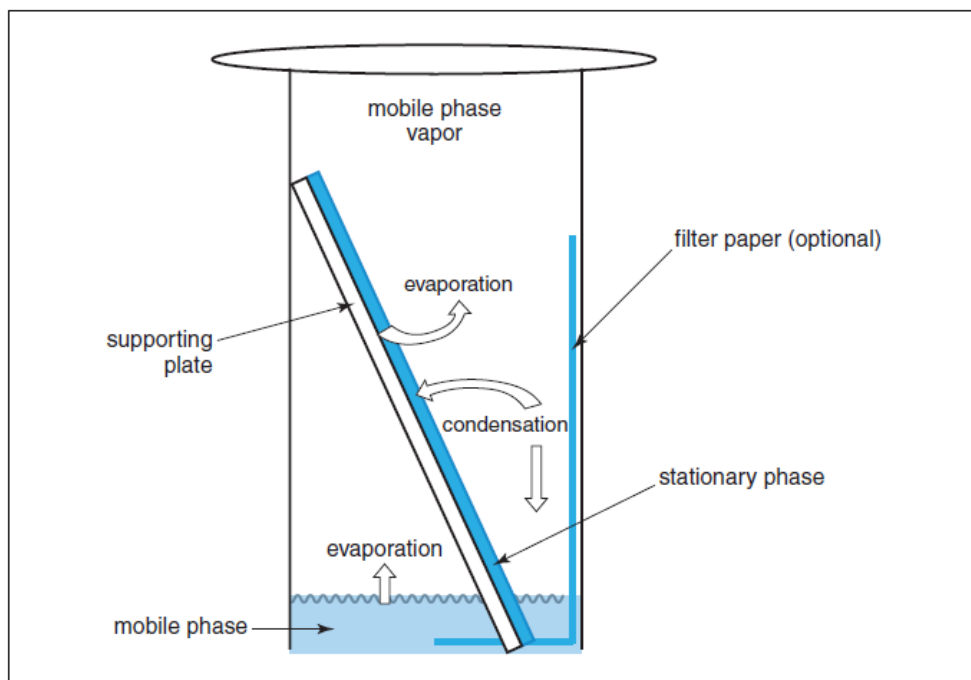


Figure 1 Schematic representation of ascending development chamber for conventional TLC (side-on view).

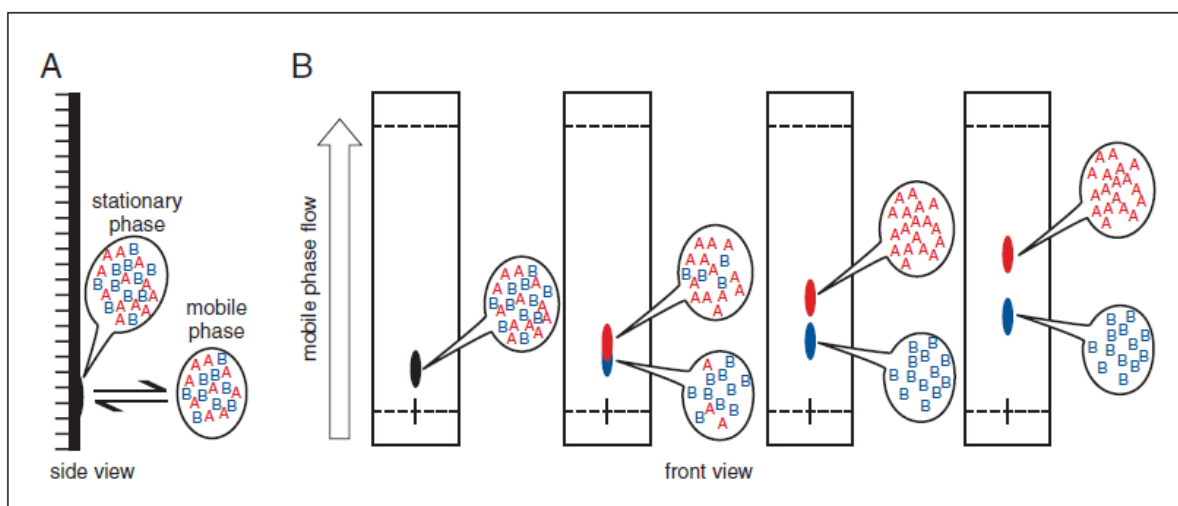


Figure 2 (A) Mixture of A and B adsorbed on the stationary phase and free in mobile phase and (B) schematic representations of the principle of separation.

STRATEGIC QUESTIONS

Before performing a TLC experiment and the subsequent analysis, the following questions should be addressed:

1. What type of TLC plate will you use regarding the backing support and coating material?
2. Which solvent system will you choose to achieve the best separation and resolution?
3. How will you handle and develop the TLC plate?
4. How will the compounds in your sample be visualized?
5. How will you solve the problems encountered during TLC experiments?
6. Are there any special techniques and tips for a successful TLC analysis?

Whether your TLC is successful, as well as the overall time required, depend on each of these decisions. The answers to these questions can be found in Strategic Planning, Protocols, and especially Troubleshooting.

STRATEGIC PLANNING

Precoated TLC Plates

Supports for stationary phases (glass, aluminum, and plastic)

Glass has been found to be a very robust support. It is rigid and transparent, and has high chemical resistance and good heat stability. The glass backing is economical (reusable). However, glass plates are relatively heavy and thick. They cannot be easily cut to desired size (see steps for handling and cutting TLC plates in the Basic Protocol, below). Because glass backing is fragile and highly susceptible to breakage, there is also a potential safety issue. Aluminum foil is preferable to all other materials for TLC plates. Compared with glass plates, foil plates are thin, lightweight, and easy to handle. They can easily be cut to desired dimensions with scissors and can be stored in a laboratory notebook. Moreover, aluminum plates have strong adsorbent layer adherence and are good for use with eluents containing a high concentration of water. However, they are not as chemically resistant as glass to reagents that contain strong acids, concentrated ammonia, or iodine (i.e., they do not tolerate long treatments in an iodine chamber). Plastic—polyethylene terephthalate (PET) film—plates are becoming less frequently used. Their advantages (thin, lightweight, easy to handle, can be easily cut, etc.) are similar to aluminum-foil plates, but their flexibility (adsorbent layer may be more susceptible to cracking) and considerably inferior heat stability are very marked disadvantages.

Adsorbent layers and stationary phases

The standard silica coating (silica 60 with a mean pore diameter of 60 Å) is the most commonly used adsorbent in TLC, although for some very sensitive substances less active adsorbents such as aluminum oxide are preferred to prevent sample decomposition. Moreover, in the early days, the use of cellulose, polyamide, and Florisil (magnesium silicate) as adsorbent agents was also described. For selection of an adsorbent, one considers the properties of the compounds to be separated: first, the solubility of the sample compounds (hydrophilic or hydrophobic); then, whether the compounds can chemically react with the adsorbent or the eluent. Based on these considerations it is recommended that:

1. for lipophilic substances: silica, aluminum oxide, acetylated cellulose, polyamide should be used;
2. for hydrophilic substances: cellulose, cellulose ion exchangers, polyamide, and reversed-phase silica should be used.

Several different types of TLC stationary phases are listed according to polarity in Figure 3. Figure 4 shows affinity of common functional groups for silica gel (approximate). Assuming that a polar adsorbent (silica gel) is used, the more polar compounds will be eluted more slowly and the more nonpolar compounds will be eluted more rapidly. The charts depicted in these figures are very useful to help predict the order of elution; however, the functional groups should always be viewed and considered within the context of a whole molecule. Clear answers come from real experiments!

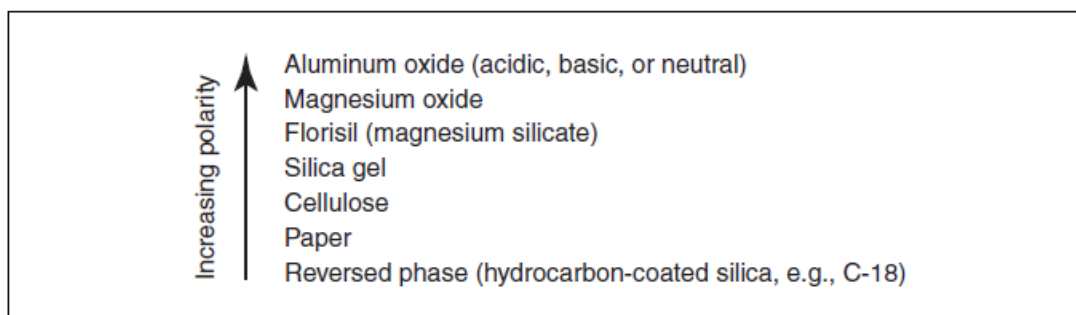


Figure 3 TLC stationary phase polarities.

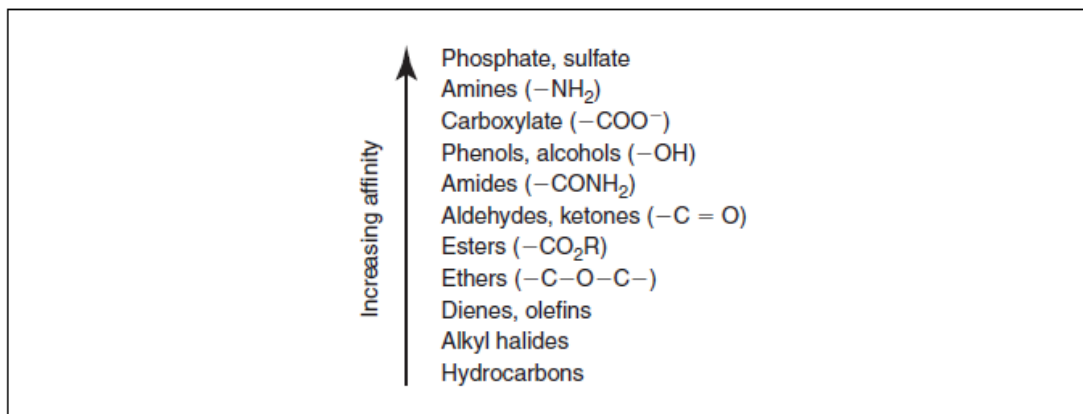


Figure 4 Affinity of common functional groups for silica gel (approximate).

Solvent System (Mobile Phase)

Finding a suitable solvent system is usually the most difficult part of TLC experiments, and solvent system is the factor with the greatest influence on TLC. Only in a few cases does the solvent consist of only one component, and mixtures of up to five components are commonly used. No matter how many components are present, the prepared solvent system must be a homogenous system with no sign of cloudiness. Three criteria are usually considered for choosing a solvent system: solubility, affinity, and resolution. The first step in solvent selection is to determine the solubility of the sample. The desired mobile phase will be able to provide the greatest solubility while balancing the sample affinity for the solvent and the stationary phase to achieve separation. Resolution is improved by optimizing the affinity between sample, solvent, and stationary phase. Most TLC solvent systems contain a polar solvent and a chromatographically less polar solvent. Figure 5 lists some common mobile phase solvents according to their polarities and elution power with silica 60 as the stationary phase (Halpaap's eluotropic series, Halpaap and Rippahn, 1976; Hahn-Deinstrop, 2006). With these solvents, there are some common combinations for organic molecules with silica gel as the stationary phase (see Troubleshooting for combinations of three solvents for very polar compounds):

Hexane (or petroleum ether)/ethyl acetate
 Dichloromethane (or chloroform)/methanol
 Pentane/ether
 Petroleum ether/acetone
 Hexane/dichloromethane
 Dichloromethane/ethyl acetate
 Ethyl acetate/methanol
 Toluene/acetonitrile
 Water/methanol (for C₁₈-reversed phase silica)
 Water/acetonitrile (for C₁₈-reversed phase silica).

The easiest way to find a starting point for development is to look up a reference for chromatography conditions of compounds with similar structure. Meanwhile, consider the affinity for the type of compound (Fig. 6.3.4), as well as the solvent strength (Fig. 6.3.5), to make adjustments. If the mobile phase has not been previously reported or determined, start with a less polar combination such as hexane/ethyl acetate and observe the separation. If the components do not move very far, try adding a greater volume or a higher ratio/percentage of the polar solvent. Always compare the separation to the previous plate. If the spots stay at the starting line of the plate, add more of the polar solvent or switch to a more polar combination such as dichloromethane/methanol. If they run with the solvent front (or $R_f > 0.8$), then add more nonpolar solvent or switch to an even less polar combination such as pentane/ether. It is common to try three to six solvent systems for the first round of method development. As a general guide, a substitution in the more polar solvent often results in a change in resolution, while a change in the less polar solvent results primarily in a change in R_f of the sample components (see Understanding Results for discussion of R_f).

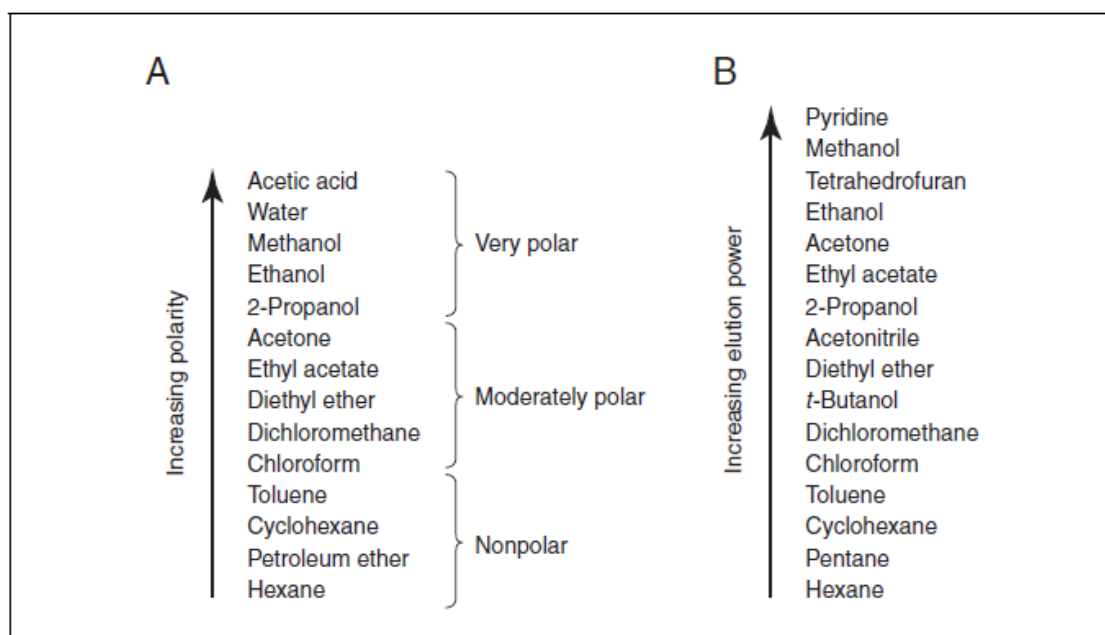


Figure 5 (A) Common mobile phase solvents listed by increasing polarity (adapted from Grace catalog for TLC accessories: and (B) elution power with silica gel as the stationary phase

SAFETY CONSIDERATIONS

1. Take extra caution when breaking scored glass TLC plates. The resulting sharp edges may cause cuts to the hands.
2. Inhaling silica gel (dust form particularly) is highly dangerous and may cause severe lung irritation. Long-term exposure may cause the lung disease silicosis. A safety mask is recommended when handling silica-gel TLC plates.
3. Many organic solvents used for developing TLC are flammable or combustible, and inhalation of their vapors is to be avoided. Some organic solvents are potentially carcinogenic, such as benzene (proven group 1 carcinogenic; should be replaced with toluene), chloroform, and dichloromethane.
4. Many reagents used in TLC staining are toxic and must be handled with care. If heating (with a hot plate or a heat gun) is required for staining, make sure all steps are carried out in a fume hood with care to avoid inhalation of any toxic or irritant smoke or vapor. Personal protections (disposal gloves, safety goggles, and masks) are required.

PROTOCOL

Basic Protocol: Principal Steps of TLC and Required Instrumentation

In this protocol, preparation of the TLC plates, preparation of the spotting capillary, spotting of the sample, development of the TLC plate, and visualization of the components are described. Necessary TLC accessories are illustrated in the associated figures with instructions for their use included in the protocol steps.

Materials

Organic sample solution: the sample for TLC can be dissolved in any compatible solvent because the solvent used to dissolve the sample will be completely dried out after the sample is spotted on the TLC (avoid high-boiling-point solvents which would make it difficult to dry the sample after spotting and cause the TLC look like a spread; see Troubleshooting) Developing solvents (CH_2Cl_2 , hexanes, ethyl acetate, methanol, etc.)

Iodine (I_2)

TLC staining reagents

TLC plates (aluminum, glass, or plastic; e.g., EMD Millipore, SORBTECH, Sigma-Aldrich)

Guillotine paper trimmer

Diamond-tipped glass cutter

Glass Pasteur pipets for capillary spotters

TLC chamber or small wide-mouth flat-bottom glass jar/bottle with a lid
 Heat gun (optional)
 UV lamp
 Iodine chamber: a screw-top glass jar with a well-fitting lid can be used as the vaporization chamber
 Filter paper
 Hot plate or TLC plate heater

Table 1 Frequently Used TLC Stains

Name	Application	Preparation
Iodine (I ₂)	Temporary stain; insert the TLC plate into the chamber and remove it after it develops a light brown color over the entire plate	To a glass bottle with cap (bottle size depends on how much stain you prepare) add 100 g of silica and 5 to 7 g of iodine crystals (no liquid). Close the cap and shake many times so that iodine is dispersed over the silica.
<i>p</i> -Anisaldehyde	Carbohydrates; heating required to stain the plate; various colors	Dissolve 18 ml of <i>p</i> -anisaldehyde in 540 ml 95% ethanol and cool the solution in an ice/water bath. Mix 30 ml of 97% H ₂ SO ₄ and 6 ml of acetic acid. Cautiously add the acid mixture to the prechilled ethanol solution dropwise at 0°C with vigorous stirring, without splashing. Store the resulting colorless solution in a – 20°C freezer before use.
Bromocresol green	Carboxylic acids yield yellow-green spots on blue background; no heating required	Dissolve bromocresol green (0.08 g) in ethanol (200 ml) to get a clear colorless solution. Slowly add 0.1 N NaOH dropwise until blue color just appears in the solution.
CAM	Universal stain; heating required to stain the plate; yields dark blue spots on light background	Slowly add conc. H ₂ SO ₄ (80 ml) to water (720 ml) under stirring followed by ammonium molybdate (40 g) and ceric ammonium sulfate (1.6 g). Stir the resulting mixture to get a clear solution.
Cerium(IV) sulfate [Ce(SO ₄) ₂]	General staining, very effective for alkaloids; should be sprayed on to the plate (not dipped) and then heated for the stain to appear as black spots on yellow-white background	15% aqueous sulfuric acid saturated with cerium (IV) sulfate
Chromic acid	General staining; yields black spots	To a cold (0°C) solution of sulfuric acid (100 ml, 20% v/v aq.), slowly add potassium chromate (2.5 g). Warm the resulting clear bright red/orange solution to room temperature and use directly.
2,4-DNP	Mainly for aldehydes and ketones; yields orange spots, no heating required	Dissolve 2,4-dinitrophenylhydrazine (6 g) in 95% ethanol (100 ml) and add water (40 ml). Stir the resulting mixture to get a clear solution, slowly add conc. H ₂ SO ₄ (60 ml), and stir to get a clear solution.
Dragendorff reagent	Unreactive amines (e.g., carbamate protected amines), alkaloids; yields orange spots, no heating required	Solution A: 1.7 g basic bismuth nitrate in 100 ml water/acetic acid (4:1). Solution B: 40 g potassium iodide in 100 ml water. Mix reagents together as follows: 5 ml A + 5 ml B + 20 ml acetic acid + 70 ml water. Spray plates; orange spots develop. Spots intensify if sprayed later with HCl or 50% water-phosphoric acid.
Ehrlich's reagent	Amines, indole derivatives, antibiotics, steroids; mild heating (lower temperature and shorter heating time; remove the heat source before the background color obscures the spots) required to stain the plate	Dissolve <i>p</i> -dimethylaminobenzaldehyde (1.0 g) in 75 ml of methanol and add 50 ml of conc. HCl.

Ferric chloride spray	Phenols	Dissolve ferric (III) chloride (1 g) in a mixture of methanol (50 ml) and deionized water (50 ml). Stir the above mixture to get a homogenous solution.
Iodoplatinate (PIP)	Alkaloids	Dissolve hexachloroplatinate (0.5 g) and potassium iodide (10 g) in deionized water (295 ml). To the above mixture add conc. HCl (27 ml). Stir the mixture 4 hr at 0°C.
Morin hydrate	General stain; fluorescently active	Dissolve morin hydrate (100 mg) in methanol (100 g) and stir to get a clear solution
Ninhydrin	Excellent stain for amines, amino acids, and aminosugars	Dissolve ninhydrin (1.5 g) in <i>n</i> -butanol (100 ml) and then add glacial acetic acid (3 ml). Ethanol can be used in place of butanol.
Potassium permanganate (KMnO ₄)	Mainly for unsaturated compounds and alcohols; alkenes/alkynes/aromatics usually stain without heating while other oxidizable groups require heating; yields yellow spots on purple background	Dissolve KMnO ₄ (1.5 g) and K ₂ CO ₃ (10 g) in deionized water (200 ml). To this add 10% NaOH (1.25 ml) and stir to get a clear solution. It will take some time for the solution to clear.
Phosphomolybdic acid (PMA)	Good general reagent; heating required to stain the plate, yields blue–dark green spots	Dissolve 12 g phosphomolybdic acid in 250 ml ethanol.
Sulfuric acid	Heating required to stain the plate; permanent charred spots are produced	5% sulfuric acid in methanol
Vanillin	Good general reagent; heating required to stain the plate, yields a range of colors	To a cold (0°C) clear colorless solution of vanillin (15 g) in absolute ethanol (250 ml), slowly add sulfuric acid (2.5 ml). Warm the resulting clear solution to room temperature and use directly. Store the excess in a refrigerator.

Handling and cutting TLC plates

For an aluminum-foil TLC plate: Place the aluminum foil TLC plate on a paper trimmer board with the coating side down (Fig 6) and use the guillotine to cut the aluminum-foil plates into desired sizes. Gently wipe the cut edges with a spatula to remove loose layer material.

Sample application (spotting the TLC plate)

The most common technique for TLC sample application is to use a glass capillary spotter.

1. The spotting capillaries are extremely small and easy to make from glass Pasteur pipets (steps 2 to 4, illustrated in Fig. 8).
 2. Heat the middle part of the Pasteur pipet over a Bunsen burner blue flame. *Hold both ends carefully, as they will get hot! Use heat-proof gloves if necessary!*
 3. When the middle part is hot enough, it will become pliable; pull both ends apart quickly and smoothly. *This will draw the middle part into a thin string-like tube.*
 4. After cooling for a few minutes, gently break the middle string-like part into 2- in. sections to provide good TLC capillary spotters. *The end should be as flat as possible, as a jagged edge will lead to unsymmetrical spots or no spots on the TLC plate.*
 5. Fill the prepared capillary spotter by quickly dipping it into the organic sample solution. *With aqueous solutions, filling will be much slower—an Eppendorf pipettor with 10- μ l tips thus recommended for applying aqueous sample solutions.*
1. Place the capillary spotter at the starting line (labeled in pencil) on the coated side of the plate vertically and carefully, to allow capillary action to draw the solution onto the plate. If more than one sample is running at the same time, make sure to properly label the plate with a pencil (Fig. 6.3.9) and use a different capillary for each sample to avoid contamination. Blow with cold or hot air to facilitate solvent evaporation of **Thin Layer** the applied samples. *To keep spots small and compact, it is better to apply a sample in several portions with intermediate drying (to avoid diffusing), instead of holding the capillary against the plate for a long time. For beginners, it is*

best to practice spotting a few times on a spare TLC strip. In this way, you will discover the capillary emptying time and have better control over the application pressure to be used with capillaries (avoiding damage to the layer).

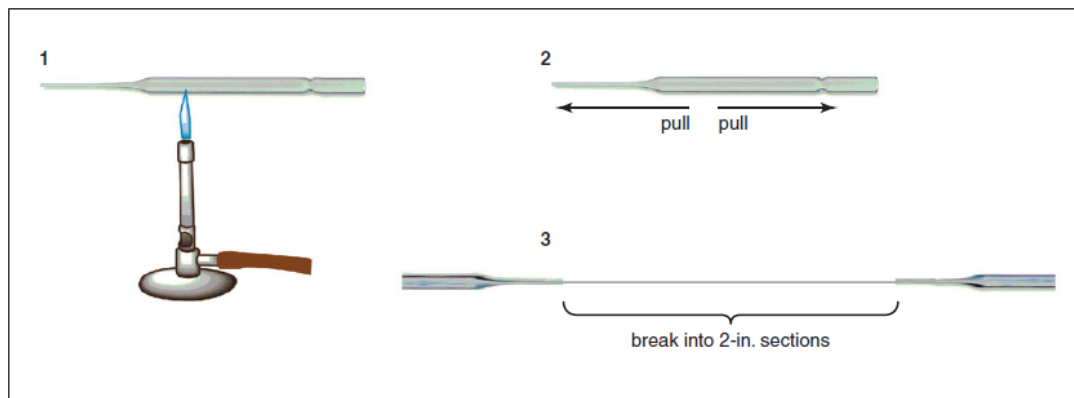


Figure 8 Making glass capillary spotter from Pasteur pipets.

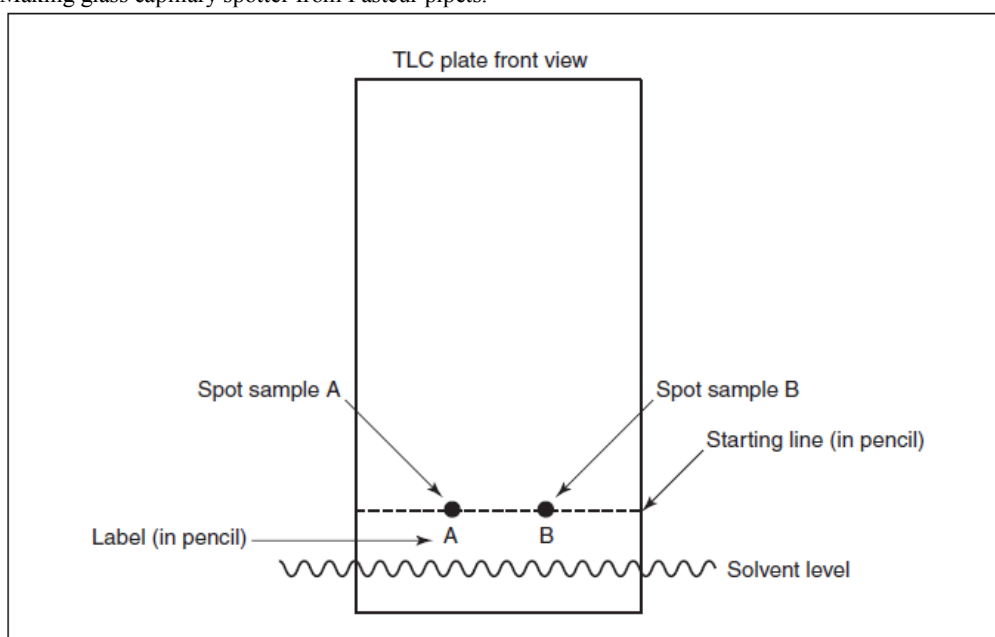


Figure 9 Spotting TLC plate.



Figure 10 Flat bottom TLC chamber.

Development of TLC plates

In most cases, ascending TLC is applied in a TLC chamber as for development once with a single solvent system (single development). Commercial TLC chambers of different sizes are readily available (Fig. 6.3.10), but usually a small wide-mouth flatbottom glass jar/bottle with a lid large enough to fit the TLC plate works just as well.

7. First, fill the chamber with development solvent to a depth no greater than 0.5 cm.

8. Use tweezers to place the TLC plate in the prepared development chamber with its back layer leaning against the chamber's inside wall, and immediately cover the chamber with the lid (Fig. 6.3.1).

Make sure that the starting line is above the solvent level (Fig. 6.3.9). Saturation of the chamber atmosphere with solvent vapor is important for TLC. In most cases, lining the chamber with a piece of filter paper (wetted with the eluent) will promote the saturation and may improve the separation and reproducibility (Fig. 6.3.1). Thus, to maintain the atmosphere in the developing chamber, it must not be opened during the development. Observe the solvent front through the side wall while keeping the chamber closed!

9. Development starts once the TLC plate is immersed; when the solvent front has reached an appropriate level (usually within 0.7 cm of the top of the plate), quickly remove the lid, take out the plate with tweezers, and mark the solvent front with a pencil.

Proper marking of the solvent front will be of benefit in the analysis of results (see Understanding Results).

10. Allow the plate to dry in a fume hood or with a heat gun (heat gently) before proceeding to the visualization step. *If certain separation cannot be achieved by a single development, multiple developments (with or without change of solvent system) may be performed: the TLC is developed two or more times with intermediate drying (also see Troubleshooting).*

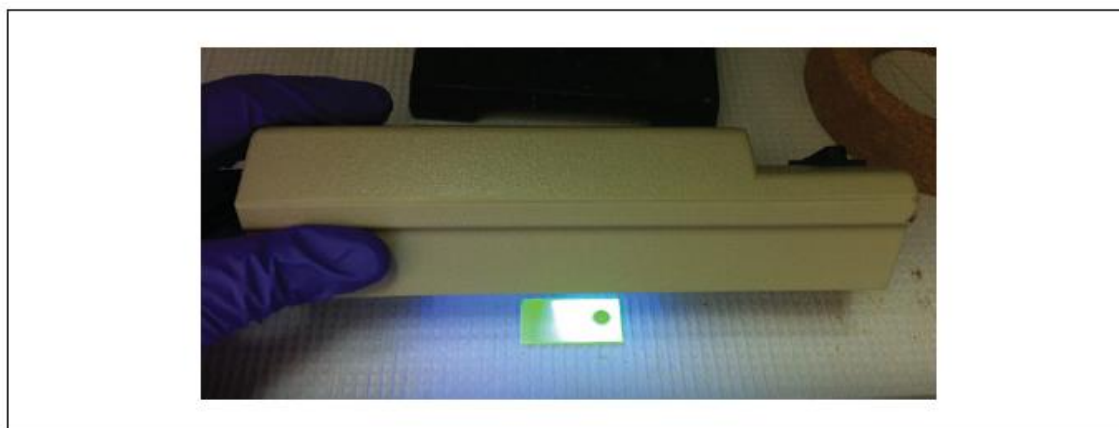


Figure 11 Visualization under a UV lamp.

Visualization

Nondestructive visualization

11a. *For nondestructive visualization with the naked eye:* Simply put the plate under a UV lamp (Fig. 11), and the compounds become visible to the naked eye. Lightly circle the spots with a pencil, so that you will have a permanent record of their location for later qualitative assignment.

Only in a few cases is the sample a dye (colored) that can be seen with naked eye. Much more often, substance visualization can be achieved under UV light, since many substances have UV absorption. TLC plates normally contain a fluorescent indicator that makes the TLC plate glow green under UV light of wavelength 254 nm (less frequently at 365 nm with a mercury lamp). Substances absorbing UV light in the respective region of wavelength will quench the green fluorescence, yielding dark purple or bluish spots on the plate (Fig. 6.3.11).

11b. *For nondestructive visualization using iodine:* Use tweezers to insert the TLC plate into the prepared iodine chamber (described in Table 6.3.1) and remove it after it develops a light brown color over the entire plate. Cover the iodine-treated TLC plates with a clean glass plate, since the color stain will eventually fade.

You may also circle the observed spots with a pencil for documentation. Iodine sublimates and will absorb to organic molecules. This method is therefore nonspecific, but usually does not cause decomposition. The organic spots on the plate will turn brown and can be easily identified. Dwell times for plates in a vaporization chamber

can range from a few minutes for detection up to 20 hr for purity tests. It should be noted that, with long dwell times in iodine vapor, aluminum TLC plates may react with iodine and become impossible to evaluate.

For stepwise dipping technique

11d. Hold the edge of the developed plate (dried) with tweezers, quickly immerse the whole plate into the staining solution, and immediately take out the plate. Close the dipping chamber with a lid immediately.

As an alternative derivatization technique, brief dipping of the TLC plates in staining reagent solutions is being used increasingly for many reasons: (1) the application of the reagent solution to the TLC layer is more homogeneous and uniform; (2) this technique gives improved reproducibility; (3) the consumption of reagent is low; (4) complex spraying equipment is not needed; (5) contamination of the workplace with reagent is minimized.

Dipping chambers/jars vary in size and shape, and dipping solutions are generally less concentrated than spraying solutions. Under certain circumstances, the dwell time in the dipping chamber should be determined experimentally.

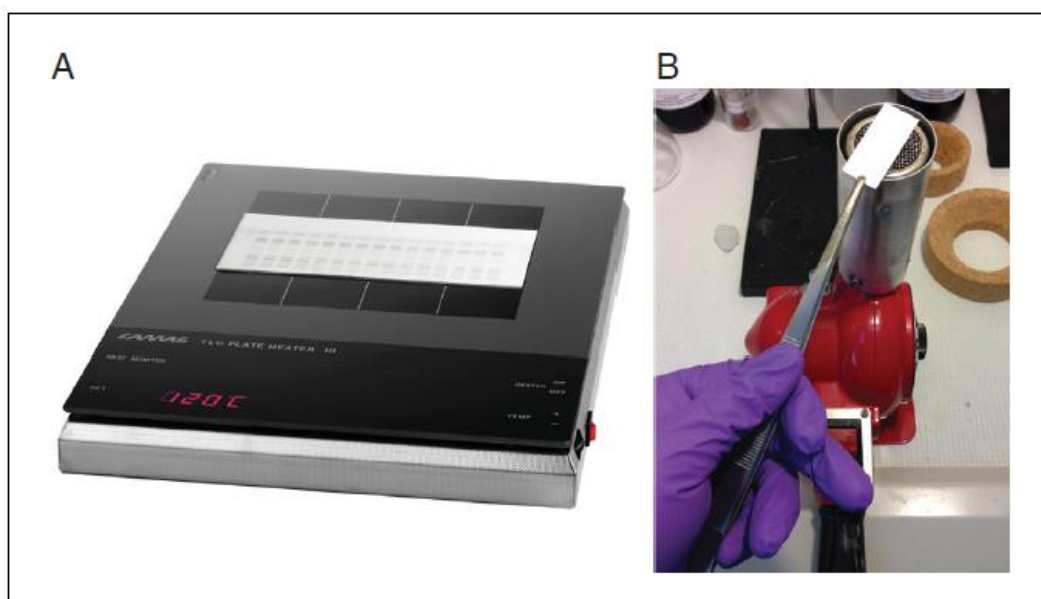


Figure 13 (A) TLC Plate Heater. (B) Heating TLC plate with a heat gun.

12d. After the dipping operation (especially before heating), carefully wipe the back of the plate with a paper towel that has been wetted with the staining solution. *The dipping chamber should always be closed with a lid after dipping to prevent evaporation of solvent.*

13. If heating is necessary, perform this on a hotplate (or a TLC plate heater, Fig. 13A), or with a heat gun (Fig. 13B).

Make sure to remove the TLC plate from the heat source once the spots are visible and before the background color obscures the spots. When glass plates are used, the spots can sometimes be seen more clearly from the glass side of the plate.

COMMENTARY

Understanding Results

Retention factor (R_f)

The analysis of TLC depends on the purpose. For most qualitative determinations, localization of substances is sufficient. This can be easily achieved by parallel runs with reference compounds (e.g., authentic sample, starting material, compound with similar structure, etc.). A practical example of a TLC analysis for monitoring the progress of a reaction is illustrated in Figure 6.3.14 (adapted from Zhao et al., 2010). The starting material, *N*-acetylglucosamine (Lane A), and the product, *N*-acetylglucosamine-1-phosphate authentic sample (Lane B), were used as controls by parallel runs with the reaction mixture (Lane C). The disappearance of the starting material spot and generation of the new product spot in the reaction mixture clearly indicate complete consumption of the starting material and completion of the reaction. An important parameter often used for qualitative analysis of TLC is the R_f value (retention factor, Fig. 6.3.15). If two spots travel the same distance or

have the same R_f value, then it might be concluded that the two components are the same molecule. However, identical R_f values do not necessarily mean identical compounds. For R_f value comparisons to be valid and reproducible R_f values to be obtained, TLC plates must be run under the same exact conditions with respect to chamber saturation, composition of solvents, temperature, etc. As shown in Figure 6.3.14, the authentic sample of *N*-acetylglucosamine-1-phosphate (Lane B) and *N*-acetylglucosamine-1-phosphate product from the reaction mixture (Lane C) have the same R_f value, providing evidence that the desired product was obtained.

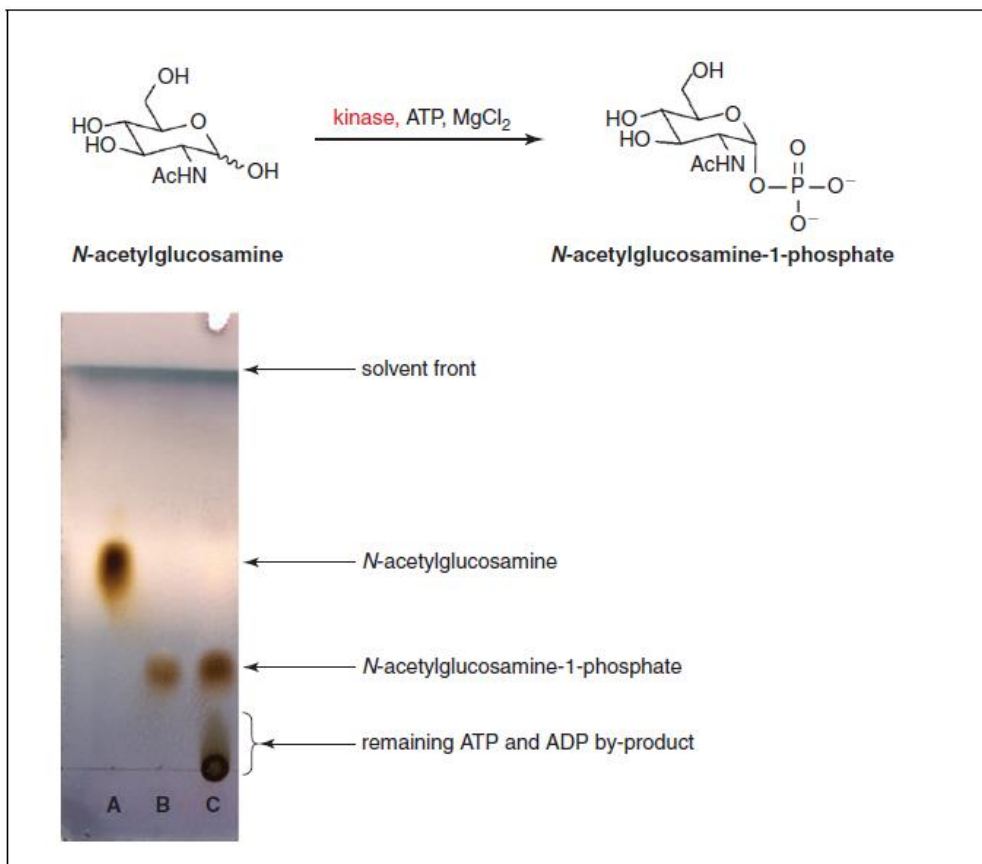


Figure 14 Use of TLC to monitor the progress of an enzymatic synthesis reaction. The developing solvent used was 2:1:1 (v/v/v) *n*-butanol:acetic acid:water for highly polar carbohydrates. The developed plate was stained with *p*-anisaldehyde stain. Lane: A, *N*-acetylglucosamine; B, *N*-acetylglucosamine-1-phosphate authentic sample; C, reaction mixture of *N*-acetylglucosamine and ATP using a kinase. The *N*-acetylglucosamine and *N*-acetylglucosamine-1-phosphate show dark/light brown on the TLC plates. The authentic sample of *N*-acetylglucosamine-1-phosphate and the *N*-acetylglucosamine-1-phosphate product have the same R_f value.

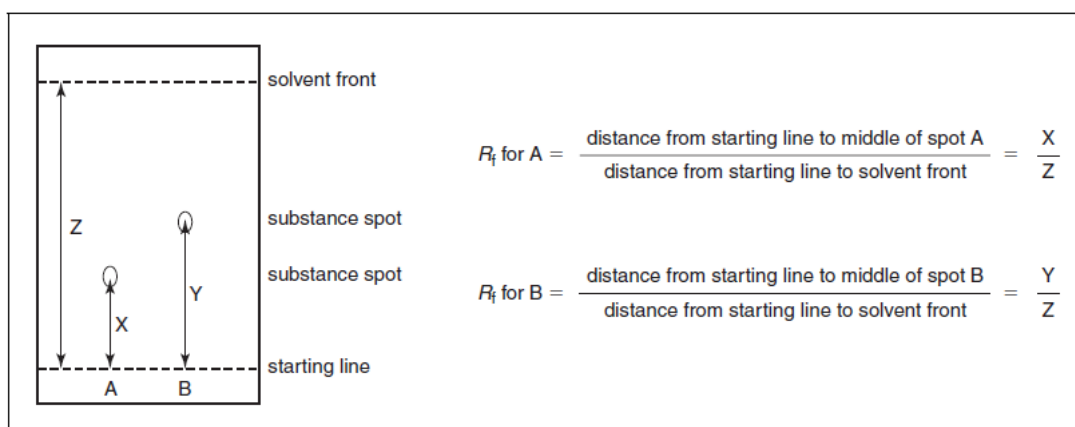


Figure 15 Calculation of R_f value. This figure shows how R_f can be measured and calculated for each spot observed on a TLC plate: the ratio of distance start–substance zone to distance start–solvent front. The R_f values are between 0 and 1. However, the optimum separation of compounds by TLC is usually achieved when R_f values are between 0.3 to 0.5. For scale-up to preparative separations, the TLC solvent system's polarity must be decreased to lower the R_f between 0.15 and 0.3.