

Stains for Developing TLC Plates

Once a TLC has been developed, it is frequently necessary to aid in the visualization of the components of a reaction mixture. This is true primarily because most organic compounds are colorless. Frequently, the organic compounds of interest contain a chromophore which may be visualized by employing either a short or a long wave UV lamp. These lamps may be found as part of a standard organic chemistry research or teaching lab. Typical examples of functional groups which may be visualized through this method are aromatic groups, α,β -unsaturated carbonyls, and any other compounds containing extensively π -conjugated systems. While exposing these TLC plates to UV light, you will notice that the silica gel will fluoresce, while any organic molecule which absorbs UV light will appear as a dark blue spot. Circling these spots gently with a dull pencil will permit an initial method for visualization. Fortunately, there are a number of permanent or semi-permanent methods for visualization which will not only allow one to see these compounds but also provide a method for determining what functional groups are contained within the molecule. This method is referred to as staining the TLC plate, and experience will allow you to determine what functional groups will appear as what color upon visualization. Following is a listing of the most commonly employed stains, the kind of compounds for which they're usually employed, and a typical recipe.

A	Note	on	TLC	Plates
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Although it should be obvious, be sure that the kind of TLC plate you are using is compatible with the stain or the conditions for its development. For instance, the inexpensive plates using a plastic polymer backing cannot be used for stains requiring heat for development. Glass backing is fine for this, but the silica gel is typically not tightly bonded to the glass, and tends to be inadvertently scraped off very easily; thus, these are not suitable for storage following development. In our group, we use aluminum-backed plates, which are less expensive than glass, are heat-impervious, the silica gel is very tightly bound to the backing, and are so thin that, if desired, a particularly spectacular plate can be taped into your lab notebook.

The Stain List

Iodine

The staining of a TLC plate with iodine vapor is among the oldest methods for the visualization of organic compounds. It is based upon the observation that iodine has a high affinity for both unsaturated and aromatic compounds.

Recipe

A chamber may be assembled as follows: To 100 mL wide mouth jar (with cap) is added a piece of filter paper and few crystals of iodine. Iodine has a high vapor pressure for a solid and the chamber will rapidly become saturated with iodine vapor. Insert your TLC plate and allow it to remain within the chamber until it develops a light brown color over the entire plate. Commonly, if your compound has an affinity for iodine, it will appear as a dark brown spot on a lighter brown background. Carefully remove the TLC plate at this point and gently circle the spots with a dull pencil. The iodine will not remain on the TLC plate for long periods of time so circling these spots is necessary if one wishes to refer to these TLC's at a later date.

Ultraviolet Light

Good for visualizing any compounds which are UV-active, particularly those with extended conjugation, aromatic rings, etc. Spot(s) must be lightly traced with a pencil while visible, since when the UV light is removed, the spots disappear.

Ceric Ammonium Sulfate

Specifically developed for vinca alkaloids (aspidospermas).

Recipe

Prepare a 1% solution of cerium (IV) ammonium sulfate in 50% phosphoric acid.

Cerium Sulfate

General stain, particularly effective for alkaloids.

Recipe

Prepare an aqueous solution of 10% cerium (IV) sulfate and 15% sulfuric acid.

Ferric Chloride

Excellent for phenols.

Recipe

Prepare a solution of 1% ferric (III) chloride in 50% aqueous methanol.

Morin Hydrate

General stain (morin is a hydroxy flavone), is fluorescently active.

Recipe

Prepare a 0.1% solution of morin hydrate (by weight) in methanol.

Ninhydrin

Excellent for amino acids

Recipe

Dissolve 1.5g ninhydrin in 100mL of *n*-butanol and then add 3.0mL acetic acid.

Dinitrophenylhydrazine (DNP)

Developed mainly for aldehydes and ketones; forms the corresponding hydrazones, which are usually yellow to orange and thus easily visualized.

Recipe

Dissolve 12g of 2,4-dinitrophenylhydrazine, 60mL of conc. sulfuric acid, and 80mL of water in 200mL of 95% ethanol.

Vanillin

Very good general stain, giving a range of colors for different spots.

Recipe

Prepare a solution of 15g vanillin in 250mL ethanol and 2.5mL conc. sulfuric acid.

Potassium Permanganate

This particular stain is excellent for functional groups which are sensitive to oxidation. Alkenes and alkynes will appear readily on a TLC plate following immersion into the stain and will appear as a bright yellow spot on a bright purple background. Alcohols, amines, sulfides, mercaptans and other oxidizable functional groups may also be visualized, however it will be necessary to gently heat the TLC plate

following immersion into the stain. These spots will appear as either yellow or light brown on a light purple or pink background. Again it would be advantageous to circle such spots following visualization as eventually the TLC will take on a light brown color upon standing for prolonged periods of time.

Recipe

Dissolve 1.5g of KMnO_4 , 10g K_2CO_3 , and 1.25mL 10% NaOH in 200mL water. A typical lifetime for this stain is approximately 3 months.

Bromocresol Green Stain

This particular stain is excellent for functional groups whose pKa is approximately 5.0 and lower. Thus, this stain provides an excellent means of selectively visualizing carboxylic acids. These will appear as bright yellow spots on either a dark or light blue background and typically, it is not necessary to heat the TLC plate following immersion. This TLC visualization method has a fairly long lifetime (usually weeks) thus, it is not often necessary to circle such spots following activation by staining.

Recipe

To 100 ml of absolute ethanol is added 0.04 g of bromocresol green. Then a 0.1 M solution of aqueous NaOH is added dropwise until a blue color just appears in solution (the solution should be colorless prior to addition). Ideally, these stains may be stored in 100 mL wide mouth jars. The lifetime of such a solution typically depends upon solvent evaporation. Thus, it would be advantageous to tightly seal such jars in-between uses.

Cerium Molybdate Stain (Hanessian's Stain)

This stain is a highly sensitive, multipurpose (multifunctional group stain). One word of caution, very minor constituents may appear as significant impurities by employing this stain. To ensure accurate results when employing this stain, it is necessary to heat the treated TLC plate vigorously (a heat gun works well). Thus, this may not be a stain to employ if your sample is somewhat volatile. The TLC plate itself will appear as either light blue or light green upon treatment, while the color of the spots may vary (although they usually appear as a dark blue spot). Typically, functional groups will not be distinguishable based upon the color of their spots; however, it would be worth while to make a list of potential colors of various functional groups as you experience variations in colors. This may permit future correlations which may prove beneficial when performing similar chemistry on related substrates.

Recipe

To 235 mL of distilled water was added 12 g of ammonium molybdate, 0.5 g of ceric ammonium molybdate, and 15 mL of concentrated sulfuric acid. Storage is possible in a 250 mL wide mouth jar. This stain has a long shelf-life so long as solvent evaporation is limited. It may also prove worth while to surround the jar with aluminum foil as the stain may be somewhat photo-sensitive and exposure to direct light may shorten the shelf-life of this reagent. It is worth while to also mention that it would be beneficial to circle the observed spots with a dull pencil following heating as this stain will eventually fade on the TLC plate after a few days.

***p*-Anisaldehyde Stain #1**

This stain is an excellent multipurpose visualization method for examining TLC plates. It is sensitive to most functional groups, especially those which are strongly and weakly nucleophilic. It tends to be insensitive to alkenes, alkynes, and aromatic compounds unless other functional groups are present in the molecules which are being analyzed. It tends to stain the TLC plate itself, upon mild heating, to a light pink color, while other functional groups tend to vary with respect to coloration. It is recommended that a record is kept of which functional group stains which color for future reference, although these types of comparisons may be misleading when attempting to ascertain which functional groups are present in a molecule (especially in complex molecules). The shelf-life of this stain tends to be quite long except when exposed to direct light or solvent is allowed to evaporate. It is recommended that the stain be stored in a 100 mL wide mouth jar wrapped with aluminum foil to ensure a long life time.

Recipe

To 135 mL of absolute ethanol was added 5 mL of concentrated sulfuric acid, 1.5 mL of glacial acetic acid and 3.7 mL of *p*-anisaldehyde. The solution is then stirred vigorously to ensure homogeneity. The resulting staining solution is ideally stored in a 100 mL wide mouth jar covered with aluminum foil.

***p*-Anisaldehyde Stain #2**

A more specialized stain than #1 (above), used for terpenes, cineoles, withanolides, acronycine, etc. As above, heating with a heat gun must be employed to effect visualization.

Recipe

Prepare solution as follows: anisaldehyde:HClO₄:acetone:water
(1:10:20:80)

Phosphomolybdic Acid (PMA) Stain

Phosphomolybdic acid stain is a good "universal" stain which is fairly sensitive to low concentrated solutions. It will stain most functional groups, however it does not distinguish between different functional groups based upon the coloration of the spots on the TLC plate. Most often, TLC's treated with this stain will appear as a light green color, while compounds of interest will appear as much darker green spots. It is necessary to heat TLC plates treated with this solution in order to activate the stain for visualization. The shelf life of these solutions are typically quite long, provided solvent evaporation is kept to a minimum.

Recipe

Dissolve 10 g of phosphomolybdic acid in 100 mL of absolute ethanol.

Occasionally, if you find it necessary to develop or investigate other staining techniques, the following references may be helpful:

- *Handbook of Thin-Layer Chromatography* J. Sherman and B. Fried, Eds., Marcel Dekker, New York, NY, 1991.
- *Thin-Layer Chromatography* 2nd ed. E. Stahl, Springer-Verlag, New York, NY, 1969.
- *Thin-Layer Chromatography Reagents and Detection Methods, Vol. 1a: Physical and Chemical Detection Methods: Fundamentals, Reagents* / H. H. Jork, W. Funk, W. Fischer, and H. Wimmer, VHC, Weinheim, Germany, 1990.
- *Thin-Layer Chromatography: Techniques of Chemistry, Vol. XIV*, 2nd ed. J. G. Kirchner and E. S. Perry, Eds., John Wiley and Sons, 1978.