Practice of Thin Layer Chromatography

Second Edition

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Preface

There are several widely used texts on thin layer chromatography (TLC) each of which is composed of two major sections: one section dealing with basics, the other dealing with specific compound class applications. In most instances, the chapters dealing with the basics of TLC do not cover their topics in enough depth and detail to enable the reader to carry out the operation described with confidence and success.

In this volume, we have attempted to describe in detail all the basic operations necessary for successful TLC in such a way that the reader may readily carry them out without hesitation. Rather than presenting complete literature reviews in each of the topic areas, we have drawn information from practical experience and from the literature describing practical methods. For best overall results, the beginning TLC practitioner should read this book from beginning to end before beginning any chromatography in order to familiarize himself or herself with the entire TLC process. This familiarity will allow each step to proceed more smoothly than might otherwise be possible.

Using this information, the worker in TLC can readily perform the desired separations in his or her own laboratory.

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Glossary of TLC Terms

absorbent	Substance that absorbs others, usually on the basis of wetting ability.
activation	The process of heating a TLC plate to drive off moisture resulting from layer preparation or adsorption from the atmosphere.
adsorbent	Substance adhering to another due to attraction between surface atoms of the two substances resulting from intermolecular forces such as hydrogen bonding, electrostatic forces, and charge-transfer forces.
adsorption chromatography	Process whereby a sample is separated by interaction between adsorptive forces of a medium (stationary phase) and a solvent (mobile phase).
alumina	Common adsorbent; Al_2O_3

argentation TLC	TLC employing silver nitrate impregnated in the layer material, usually silica gel. This impregnation changes the separation characteristics of the silica gel.
ascending chromatography	Chromatography in which the mobile phase moves upwards in the medium.
band	Chromatographic zone; region where the separated substance is concentrated.
bed	A column or layer of porous material containing the stationary phase, the interstices being filled with mobile phase.
CC	Column chromatography.
cellulose	Common medium for separation on a TLC plate.
chamber	Tank, jar, or vessel in which chromatographic separation takes place.
chamber saturation	Equilibration of the chamber or tank with mobile phase before the plate is placed into it.
chromatogram	A series of separated bands or zones in or on the stationary phase. The end product of the chromatography process.
chromatographic solvent	Solvent or mixture of solvents used as the mobile phase.
chromatographic system	Combination of the solvent, the sorbent, and components of the sample mixture. The interactions of the system determine the selectivity of the separation.
chromatography	A method of analysis in which the flow of a mobile phase (gas or liquid) promotes the separation of substances by differential migration from a narrow initial zone in a sorptive medium.

chromatoplate	A thin layer plate; a layer of sorbent coated on a solid support such as glass, aluminum, or plastic.
continuous development	Development occurring over a distance that is usually greater than one plate length. Development is often expressed as a function of time rather than distance.
deactivation	The process of making the chromatographic layer less active to decrease its separation capabilities. Usually done with water.
densitometry	Measurement of a zone on a layer with an instrument that determines the optical density of the zone.
descending chromatography	Chromatography in which the mobile phase moves downwards in the sorptive medium.
destructive detection	A detection process that changes the chemical nature of the substance being detected in an irreversible manner. Sulfuric acid charring is one example.
detection	The process of locating a separated substance on a chromatogram, whether by physical methods, chemical methods, or biological methods.
developing solvent	Mobile phase.
development	The movement of mobile phase in the chromatogram to effect separation.
diatomaceous earth	A naturally occurring fine white powder, formed from the skeletons of microscopic marine organisms. Also called kieselguhr.
distribution coefficient	$k = \dfrac{\text{amount of solute per unit of stationary phase}}{\text{amount of solute per unit of mobile phase}}$

eluent	Solvent that removes a sample from a medium.
eluotropic series	Series of solvents or solvent mixtures arranged in order of eluting power.
elution	Removal of a solute from a sorbent by passage of a suitable solvent.
flat-bed chromatography	Common term for thin layer or paper chromatography occurring in a single plane. Sometimes called planar chromatography.
front	The visible boundary at the junction of the mobile-phase wetted layer and the "dry" layer. If a trough chamber is used with equilibration for development, the "dry" layer can contain amounts of the mobile phase.
GC (GLC)	Gas (liquid) chromatography.
gradient elution	Development using a solvent system whose composition is continuously changing to effect separation; normally done to increase the strength of the eluent.
gradient TLC	Separation on a sorbent layer that has changing characteristics, that is, a gradient, from one portion of the layer through an adjoining portion of the layer.
HPLC	High pressure liquid chromatography or high performance liquid chromatography.
HPTLC	High performance thin layer chromatography.
hR_f	$100 \times R_f$
impregnation	Loading of the sorbent with a liquid or a solid to change the chromatographic behavior of the layer. An example is $NaNO_3$ impregnated silica gel.

in situ	Occurring in place, e.g., on the thin layer.
ion exchange	Process whereby ions of the same charge replace each other in a given phase. In chromatography, it usually refers to situations where the stationary phase is made of an ionic polymer, which can be a synthetic resin or a specially treated mineral.
IR	Infrared.
kieselguhr	See "diatomaceous earth."
migration	Travel of sample in the medium in the direction of the mobile phase.
mobile phase	The moving phase (solvent or gas) of a chromatographic system.
MS	Mass spectrometry.
multiple chromatography	Chromatography repeated a number of times using the same or different mobile phases.
nondestructive detection	Detection of a substance on a chromatogram by a process that will not permanently change the chemical nature of the substance being detected. Visualization with iodine vapor is one example of a nondestructive method.
origin	Point where sample is applied.
partition	Divide or distribute between.
partition chromatography	Process in which sample is separated by partition between two liquid phases or between a gas and a liquid. One liquid is stationary while the other is mobile.
partition coefficient or ratio (K_d)	Ratio of concentration of solute after partition between two immiscible phases:

	$K_d = C_s/C_m$, where C_s and C_m are the concentration in the stationary and mobile phases, respectively.
PC	Paper chromatography.
PLC	Preparative layer chromatography. Used for the separation of larger amounts of substance than are normally separated with regular, analytical TLC. Normally a thicker layer (500-2000 μ) or sorbent is employed than in TLC.
PMD	Programmed multiple development. The repeated development of a TLC plate with the same mobile phase in the same direction for gradually increasing distances.
polar	Highly charged, or with uneven electrical charges. Degrees of solubility in water can be used as a measure of polarity. In organic chemistry a polar molecule is one with a large dipole moment. In chromatography a polar molecule is one whoe distribution coefficient favors the polar phase. Affinity of substances for polar solvents depends on their dipole moments and their molecular volumes. It is clear that polarity in the strict sense is not always synonymous with solubility.
precoated plates or sheets	Commercially available thin layer plates or sheets ready for use in TLC.
resolution	The degree of separation between two substances expressed as. $Rs = \frac{1}{4}(\alpha - 1)\sqrt{N}\ (k'/k'+1)$, where α is the separation factor or the ratio of the capacity factors between two solutes k_1/k_2; N is the number of theoretical plates in the

	sorbent bed; k' is the average of k_1 and k_2. The capacity factor k is the equilibrium ratio of total solute in the stationary phase to total solute in the mobile phase.
reversed-phase	Chromatography on a sorbent impregnated with a nonpolar and nonvolatile liquid as a stationary phase. Separation is effected by a polar mobile phase.
R_f value	A ratio: the distance from the origin to the center of the separated zone divided by the distance from the origin to the solvent front.
R_m value	$\text{Log}\ (\frac{1}{R_f} - 1)$
sandwich chamber	Developing chamber formed from the plate itself, a spacer, and another nonlayered cover plate that stands in a trough containing the mobile phase.
secondary front	An additional solvent (mobile phase) front, lower than the primary solvent front. Occurs because components of the mobile phase have demixed and migrated apart from the other components.
silica gel	Silicic acid. The most widely used sorbent for TLC. Also used in column chromatography.
solvent	Liquid used for mobile phase. Not identified *a priori* with mobile phase.
solvent front	The forwardmost point of the mobile phase during development.
sorbent	A generalized term for the chromatographic stationary phase in which the nature of the force (adsorption, ion exchange, or reversed phase) is not specified.

starting point line	Position on chromatogram where the sample is applied. Usually 10-20 mm from the bottom of the plate. Also called the origin.
stationary phase	The phase of the chromatographic system that is made up of the surface of an adsorbent or liquid held by the support of a partition or gel system.
stepwise elution	Development using an eluent whose composition is changed using discontinuous, stepped gradients, in contrast to *gradient elution*.
support	The sheet of glass, plastic, or aluminum upon which the TLC sorbent is coated. Gives physical strength to the layer.
tailing	Incomplete separation of zones, often resulting in elongation of a zone.
TLC	Thin layer chromatography (chromatogram).
TLG	Thin layer gel chromatography employing a gel, such as Sephadex, coated on a glass plate for the separation of molecules predominantly according to their size.
two-dimensional chromatography	Successive development of a chromatogram in directions orthogonal to each other with the same or different mobile phase.
two-dimensional development	A two-step development technique in which a plate is first developed in one mobile phase, then dried, turned through 90°, and developed in a second, different mobile phase.
UV	Ultraviolet light.

zone (also spot or band)	The distribution of the solute or separated compound on the stationary phase before, during, and after chromatography.

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