Editorial

Thin-Layer Chromatography

Thin-Layer Chromatography (TLC) is a chromatographic technique widely used for qualitative analysis of organic compounds, isolation of the individual compounds from multicomponent mixtures, quantitative analysis, and preparative-scale isolation applied in phytochemistry, drug research, medicinal chemistry, environmental control, quality control of various products etc. In many cases, it outperforms the other chromatographic techniques. Firstly, there is a multitude of the chromatographic systems that are rarely applied in HPLC but can be applied in TLC. Many kinds of TLC and HPTLC precoated plates are commercially available [e.g., those with the inorganic adsorbent layers (silica, alumina); organic layers (polyamide, cellulose); organic, polar covalently bonded modifications of the silica matrix (diol, cyanopropyl, aminopropyl); and the organic, nonpolar bonded stationary phases (RP2, RP8, RP18) with different densities of coverage of the silica matrix]. Sorbents applied in TLC have the different surface characteristics and different physicochemical properties. Moreover, there is a wide choice of mobile phases that can be used to separate mixture components, belonging to various selectivity groups and, thus, having different properties as proton donors, proton acceptors, and dipoles. In TLC, UV absorption of the mobile phase solvents does not play a significant negative role in detection and quantitation of the analytes, because the mobile phase is evaporated from the plate prior to the detection. High viscosity of a solvent can be viewed as a sole property limiting its choice as a mobile phase component. These plate and mobile phase characteristics allow a choice from among an unparalleled abundance of TLC systems that offer a broad spectrum of separation selectivities, which is particularly important when complex mixtures have to be separated.

Another advantage of TLC is that each plate is used only once, and there is no fear of deactivation of adsorbent in multiple runs. It allows simpler sample preparation methods compared to column techniques. Highly sorbed materials in analyzed samples can be left behind in a column and interfere in the analysis of subsequent samples. Multiple samples can be analyzed at the same time on a single plate, reducing the time and solvent volume used per sample; the processing of standards and samples on the same plate leads to advantages in the accuracy and precision of quantitation by densitometry.

TLC enables usage of numerous special development techniques. Most separations are carried out by a capillary flow development with a single mobile phase (isocratic) in the ascending or horizontal configuration. Gradient elution with stepwise variations in mobile phase composition, which is widely applied in HPLC, is also used in TLC. Besides, there are the following special modes of developing a chromatogram: unidimensional multiple development (UMD), incremental multiple development (IMD), gradient multiple development (GMD), and bivariant multiple development (BMD). Moreover, the circular and anticircular development methods can also be applied. Multiple development consists of repeated development of the chromatogram with a given mobile phase of constant composition over the same development distance (UMD) or by the stepwise increase in the development distance (the increment in the development distance is kept constant) (IMD) and with drying the plate between the individual development runs. It results in narrowing of the spots or zones and improved resolution. In GMD, each step of the repeated chromatogram development is performed with a mobile phase of different composition (enabling gradient development) with steady development distance, enabling the analysis of complex mixtures spanning a wide polarity range. BMD involves a stepwise change both of the development distance and the mobile phase composition. With use of a special chamber and computer program, an improved version known as Automated Multiple Development (AMD) can be applied, with the distance of the development increasing and the mobile phase strength decreasing at each step. In the circular and anticircular development modes, the mobile phase migrates radially from the center to the periphery or from the periphery to the center, respectively. It enables better resolution of analytes with lower R_F values by means of circular chromatography than by linear chromatography, and the advantage of the anticircular mode is that it allows better resolution of compounds with higher R_F values.

TLC is also the easiest technique with which to perform multidimensional (i.e., two-dimensional) separations. One plate can be developed with mobile phase 1 and after drying the plate can be developed with mobile phase 2 at right angles (perpendicular or orthogonal direction); mobile phase 2 has different selectivity characteristics compared to mobile phase 1. In this way, complete separation of very complex mixtures can be achieved. Particularly valuable separation results can be achieved when using various mobile phase systems to benefit from different separation mechanisms. On cellulose one can avail adsorption mechanism with nonaqueous mobile phase and partition mechanism with aqueous eluent; on polar chemically bonded stationary phases nonaqueous eluents to achieve the adsorption mechanism of retention and the aqueous eluents to achieve the reversed-phase mechanism. Shifting from the adsorption to the partition mode causes marked differences in the separation selectivity. Multidimensionality can be also realized on different layers (grafted layers) and various eluents.

Spots or zones must be detected and localized. If the zones are not naturally colored or fluorescent, or do not absorb 254 nm UV light so they can be viewed as fluorescence-quenched zones on special F-plates containing a fluorescent indicator. Also detection

reagents can be applied by spraying or dipping, sometimes followed by heating. This derivatization is mainly used in the post-chromatographic mode for localization of the separated component zones on the layer. Very often the universal reagents are used, such as iodine vapors or sulfuric acid which can locate almost all of the existing organic compound classes. Selective derivatizing reagents can be used for individual or group identification of the analytes. For example, the Dragendorff's reagent ($KBiI_4$) is used for identification of heterocyclic bases (e.g., of alkaloids); ninhydrin for identification of the compounds containing the amino group in their structure (e.g., of the amines and amino acids); and 2-(diphenylboryloxy)-ethylamine + polyethylene glycol (PEG) is used for identification of polyphenols. TLC plates can be documented by videoscans or photographs.

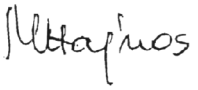
TLC coupled with densitometry enables detection of the spots or zones through scanning of the chromatograms with UV–vis light in the transmission, reflectance, or fluorescence mode. Through a comparison of a signal obtained with that for the standards processed with comparable chromatographic conditions, densitometric measurements can be used for quantitative analysis of the components contained in the mixtures. With multi-wavelength scanning of the chromatograms, spectral data of the analytes can be directly acquired from the TLC plates and can further be compared with the spectra of the analytes from the software library. Thus, a densitometer with a diode array detector enables direct (in situ) identification of the analytes. Other possibilities to identify analytes are offered by an on-line coupling of TLC with Fourier transform infrared spectrometry, mass spectrometry, etc.

Further, it is worth noticing that TLC coupled with bioautographic detection of microbiologically active compounds can be successfully applied in the analysis of the plant extracts. Especially suitable for this purpose is direct bioautography, which uses microorganisms (e.g., bacteria or fungi) growing directly on a TLC plate with the previously separated mixtures of the plant extracts. In this procedure, antibacterial or antifungal compounds appear as clear spots (i.e., without microorganisms growing on them) on an intensely colored background. This approach can be used as an additional analytical option in screening of biological samples, as a standardization method for medicinal plant extracts, and as a special and selective detection method.

Additionally, special instruments enable use of the forced-flow migration of mobile phase. Overpressured-layer chromatography (OPLC) makes use of a pump that feeds the sorbent bed with mobile phase at a selected flow rate. Rotation planar chromatography (RPC) uses centrifugal force in order to obtain an analogous effect. Electroosmotically driven TLC makes use of electroosmotic flow to force mobile phase across a layer. All of these forced-flow methods provide a constant flow rate of the mobile phase; the linear profile of the flow and elimination of vapor phase from the system improves system efficiency and peak resolution.

The advantages of TLC are particularly important with very complex mixtures of the structurally differentiated chemical compounds. Such mixtures very often contain polar and/or nonpolar ballasts, apart from a fraction of analytes. This latter fraction often contains structural analogues of a similar physicochemical properties. Isolation of a fraction of interest from such a mixture requires a complicated procedure, usually liquid-liquid and/or solid-phase extraction. TLC enables separation of a crude extracts without an earlier purification. For example, in a normal phase system a nonpolar fraction moves with the mobile phase front (or it can be prewashed with a nonpolar mobile phase prior to the development of a chromatogram) and the polar fraction remains strongly retained near to the origin; then the fraction of interest is separated in the central part of the chromatogram.

Summing up, TLC is one of the principal separation techniques. It can be used in a search for the optimum extraction solvents, for identification of known and unknown compounds, and —what is at least equally important—for selection of biologically active compounds. TLC also plays the key role in preparative isolation of compounds, in purification of the crude extracts, and in control of the separation efficiency of the different chromatographic techniques and systems. TLC has many advantages in chemistry, pharmacy, phytochemistry etc. research and development. These include single use of stationary phase (no memory effect), wide optimization possibilities with the chromatographic systems, special development modes and detection methods, storage function of chromatographic plates (all zones can be detected in every chromatogram by multiple methods), low cost of the routine analysis, and availability of purification and isolation procedures.



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