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Review

High performance stationary phases for planar chromatography☆

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ABSTRACT

The kinetic performance of stabilized particle layers, particle membranes, and thin films for thin-layer chromatography is reviewed with a focus on how layer characteristics and experimental conditions affect the observed plate height. Forced flow and pressurized planar electrochromatography are identified as the best candidates to overcome the limited performance achieved by capillary flow for stabilized particle layers. For conventional and high performance plates band broadening is dominated by molecular diffusion at low mobile phase velocities typical of capillary flow systems and by mass transfer with a significant contribution from flow anisotropy at higher flow rates typical of forced flow systems. There are few possible changes to the structure of stabilized particle layers that would significantly improve their performance for capillary flow systems while for forced flow a number of avenues for further study are identified. New media for ultra thin-layer chromatography shows encouraging possibilities for miniaturized high performance systems but the realization of their true performance requires improvements in instrumentation for sample application and detection.

Contents

1. Introduction

In most forms of chromatography both reliable and consequential relationships have been developed between theory and the operating characteristics of the separation media [1]. These interconnections are symbiotic in that the predictions from theory establish the goals for improving existing media and physicochemical studies of different media provide the means to test, modify and improve existing theory. Over time these two aspects of chromatographic evolution converge and further developments focus on narrower issues associated with the particular properties of a few compounds. Although developments in column liquid chromatography have not ceased, witness for example the recent introduction of superficially porous particles and instrumentation for operation at pressures around 1 kbar [2–4], these developments are simply accomplishments confidently predicted by theory and confirmed by advances in material design and engineering practice. Although planar chromatography predates modern liquid chromatography a reliable and consequential relationship between theory and sepa-

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ration performance has yet to develop. The myriad of reasons for this break in the normal cycle of technological evolution in separation science is discussed in this review. When the cycle is broken advances occur by an intuitive mechanism, but they still occur so long as the field of study remains active. In this article we will present a contemporary picture of these intuitive advances in planar chromatography as they pertain to the kinetic performance of layers.

Important milestones in the evolution of high performance stationary phases for planar chromatography were the early standardization of conditions for the preparation of plates for thin-layer chromatography (TLC) by Stahl [5]; the general phase out of self-made layers with the introduction of pre-coated TLC plates around 1966; the redefinition of layer performance and the penetration of instrumentation into the practice of thin-layer chromatography with the launch of pre-coated high-performance thin-layer chromatography (HPTLC) plates in about 1975 [6–8]; the introduction of pre-coated chemically modified layers in about 1978, which facilitated an expansion in the range of applications suited to thin-layer chromatography [7–10]; the introduction of layers prepared from spherical particles around 1990 [11,12]; and more recently the introduction of ultra thin-layer chromatography (UTLC) based on monolithic films [13] or microfabricated structures [14]. In this article we will focus on the more recent developments in layer characteristics and attempts to define and optimize their structures, carrying forward only those aspects of earlier studies required to establish the improvements made. Specialized layers, such as those used for chiral separations [15–17] and those prepared by impregnating pre-coated layers with reagents to enhance specific separations [1,18,19] are not discussed here. Significant contributions to the theory of thin-layer chromatography are summarized in Refs. [1,18–25] and only sufficient background to understand the main points of this article as it applies to the characterization of high performance layers will be discussed.

2. Plate height for stabilized particle layers

The common measure of band broadening for chromatographic separations is the plate height and its relationship to the physical properties of the separation system is interpreted by models such as the van Deemter equation, Knox equation, kinetic plots, etc. [1,3]. The basis of these approaches is the observation of changes in peak widths with variation in mobile phase velocity. For column chromatography these experiments are reasonably straightforward and provide considerable detail of the kinetic performance of the stationary phase. The equivalent experiments in planar chromatography are more difficult to perform and interpret and are affected by a wider range of experimental parameters that are more difficult to control within defined ranges when using capillary flow (Table 1). As a basis for discussion we can start by considering the experimental difficulties in measuring the plate height and controlling the mobile phase velocity in planar chromatography.

2.1. Experimental measurements

Samples are typically applied to layers as bands or spots that increase in size during the development process. For fine-particle layers the migrated zones are generally symmetrical and can be fit to a Gaussian peak shape model. Zones with distinct tailing are unsuitable for plate height measurements. This can be due to specific solute–stationary phase interactions with slow kinetics and disqualifies that compound for use when the purpose is to establish a general property of the stationary phase. It can occur because of inadequate layer preparation (inhomogeneous bed) or unsuitable characteristics of the stationary phase (kinetic heterogeneity of sorption interactions or diffusion properties). These are potential issues for self-made plates or new sorbents but should only be occasional problems for pre-coated layers and conventional sorbents.

Table 1
Parameters affecting the observed plate height in planar chromatography with capillary controlled flow of the mobile phase.

System property	Experimental parameters
Measurement of zone widths	Vertical distribution of sample in the layer Secondary chromatography during drying of the layer Linearity of detector response Relative size of sample application zone Reshaping of sample application zone at the start of development Absolute distance between the solvent entry position and the sample application zone Sample diffusion coefficients Sample overload
Mobile phase velocity	Variable and a function of the solvent front migration distance Varies with the saturation grade of the development chamber Affected by solvent demixing and localized unsaturated solvent flow Varies with the extent of wetting of the stationary phase Varies with the viscosity and surface tension of the mobile phase Varies with particle size distribution of the layer Varies with layer thickness

For symmetrical zones the observed plate height is calculated from the migration distance Z_S of a zone and the standard deviation for the Gaussian model for the zone profile σ_{chrom} (the standard deviation is often replaced by a specific measurement of the zone width at some fraction of the peak height as a surrogate measurement of the standard deviation).

$$H_{obs} = \frac{\sigma^2_{chrom}}{Z_S} \quad (1)$$

Although planar chromatography is generally performed as an open bed technique, allowing the zones to be visualized directly, measurements of zone dimensions are not straightforward. The eye functions as a logarithmic integrator with variable sensitivity and is not a suitable detector for estimating the position of zone boundaries for colored samples leading to high uncertainty in the estimate of σ^2_{chrom}. Such measurements are questionable at best and cannot be supported for the determination of the kinetic properties of layers [26]. Zones immobilized in the stationary phase can be converted into a chromatogram with signal as the vertical axis and migration distance as the horizontal axis using optical scanning densitometry [19,26,27]. Peak characteristics are now easily determined by software but a general problem arises from the vertical distribution of the sample in the layer [27–30]. Measurements by scanning densitometry or image analysis are typically made by reflection. The observed signal originates predominantly from the portion of the sample close to the surface with decreasing contributions from sample portions at greater distances from the surface. After development the removal of solvent by evaporation causes changes in the vertical profile of the zone resulting from secondary chromatography [28]. Little is known about the sample depth profile and its vertical homogeneity and the view from the surface may not represent the true sample distribution within the zone. This does not disqualify densitometric measurements for plate height measurements. These measurements are repeatable when adequate control over the other experimental variables is implemented and are not subjective as are visual measurements. This is not the same, however, as saying they are correct in absolute terms,