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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,

since the vertical sample distribution in the zone is unknown. Since optical scanning densitometry is the method of choice for recording thin-layer chromatograms with stabilized particle layers there is correspondence between how separations are typically recorded and modeling of layer properties. It should also be noted that the relationship between optical density and sample amount in scanning densitometry is non-linear with a pseudolinear region of the calibration curve from low to intermediate sample amounts. For simplicity plate height measurements should be made within the pseudolinear range otherwise corrections are required for the differences in response characteristics of individual zones.

When considering the meaning of H_{obs} and its comparison with column values important differences in the typical experimental protocol need to be considered. In column chromatography all sample zones travel the same distance and are distinguished by the different times they spend in the chromatographic system. The value for H_{obs} can be considered a system property with a constant value for solutes with similar physicochemical properties. This makes it a useful parameter for comparing system performance and modeling system properties. In thin-layer chromatography the sample components are separated in space (differences in migration distance) and have a constant separation time. In this case, the value for H_{obs} is dependent on the migration distance, and therefore the mobile phase velocity generated by capillary flow, and is an averaged value of the individual plate heights for all plates contacted by the sample zone. For capillary flow conditions, commonly used in thin-layer chromatography, the individual plate height values are not constant either. Consequently, H_{obs} is a complex function of the system properties and more difficult to model as a function of system properties than is the case for column chromatography.

To include the migration distance in the plate height expression Eq. (1) is easily modified [19–21,26]:

$$H_{obs} = \frac{\sigma^2_{chrom}}{R_F(Z_f - Z_0)} \quad (2)$$

where R_F is the retardation factor (the fraction of the mobile phase migration distance traveled by the sample zone measured from the sample origin), Z_f is the solvent front migration distance from the solvent entry position, and Z_0 is the distance from the solvent entry position to the sample application zone. The value for H_{obs} varies inversely with R_F and if H_{obs} values are to be compared for different systems then the values need to be determined for the same R_F value. This can be quite difficult to achieve for systems with different sorption characteristics using the same mobile phase conditions. To circumvent this difficulty H_{obs} values are usually quoted for an R_F of 0.5 or 1, as reference values. With $R_F = 1$, H_{obs} is hypothetical, corresponding to the plate height for a sample zone moving with the solvent front, and by definition, having no attractive interactions with the stationary phase. It can be considered an inflated value or upper limit for the system, since all separated substances have R_F values less than 1 in real separations and are only influenced by those plates through which they migrate.

As well as the effect of the migration distance on H_{obs} the dimensions of the sample application zone has to be considered. The standard deviation for the sample zone, σ^2_{obs}, is made up of three (assumed) independent contributions [1,7,19,31]:

$$\sigma^2_{obs} = \sigma^2_{chrom} + \sigma^2_{SA} + \sigma^2_{INS} \quad (3)$$

where σ^2_{SA} is the variance of the sample application zone and σ^2_{INS} is the contribution measured as variance associated with the operating characteristics of the densitometer, and is generally small (or assumed to be small as it is rarely measured) compared with the other contributions from the separation system and the sample application zone. Using suitable instruments sample application zones can be maintained at about 1 mm in the development direction while separated sample zones are expected to be <6 mm using pre-coated HPTLC layers. Thus, in practice σ^2_{SA} is never negligible compared with to σ^2_{chrom}. While the dimensions of the sample application zone prior to development can be determined by scanning densitometry this does not provide the correct value for σ^2_{SA} [32,33].

At the start of the development process the mobile phase encounters the lower boundary of the sample zone pushing it forward before the top portion of the zone is reached by the advancing solvent front. This effectively reshapes and concentrates the sample zone before migration commences. In addition, the sample requires a finite time to be fully solvated by the mobile phase causing expansion of the sample zone. At the advancing solvent front boundary the mobile phase is unsaturated and some pores holding sample will be filled more slowly, delaying displacement of adsorbed sample from the sorbent surface. In addition, localized solvent saturation may limit the rate of solute dissolution in the mobile phase. As a consequence, there is no accurate or consensus method for measuring the dynamic σ^2_{SA} value, although it is always significant for plate height measurements, especially for systems of high efficiency (compact zones) and for solutes with small R_F values. Three approximate approaches are generally used: (1) the variance for the dried sample application zone prior to development is adopted as a surrogate for σ^2_{SA}; (2) the rest diffusion value according to the method of Kaiser is selected [6,26]; or in a separate experiment, the sample zone is migrated a short distance (a few mm) from its origin and the dimensions of the migrated zone is adopted as a surrogate for σ^2_{SA} [32]. The method of Kaiser is based on the generally observed linear relationship between the peak width at half-height and the zone migration distance for substances with similar diffusion coefficients but different interactions with the stationary phase (chosen to produce a suitable range of R_F values). The peak width at half-height, b_s, for any zone migration distance is given by:

$$b_s = b_0 + mR_F(Z_f - Z_0) \quad (4)$$

where b_0 represents the rest diffusion value for the sample application zone, and is calculated, as is the slope, m, by linear regression. The real plate height (a term introduced by Kaiser) is then given by

$$H_{real} = \frac{(b_s - b_0)^2}{5.54R_F(Z_f - Z_0)} \quad (5)$$

Since b_s is a continuous function of R_F, H_{real} can be calculated for any R_F value independent of whether a compound in the chromatogram is located exactly at the required position. The three methods of correcting for σ^2_{SA} are compared in Fig. 1 for an HPTLC silica gel layer. The correction of H_{obs} for the contribution of σ^2_{SA} is about 5–60% and depends on the development distance and Z_0. For TLC layers corrections of 5–20% are more typical. The general shape of the three plots in Fig. 1 is similar but the vertical position of each plot on the H_{obs} axis is quite different. The location of the minimum plate heights on the migration distance axis is roughly the same in each case. Thus, it is important when comparing plate height values to always make those comparisons within the framework of the same calculation method. Since it cannot be established that any of the three methods is correct in its calculation of the true plate height a general recommendation cannot be made.

The value of the plate height is strongly dependent on Z_0, the distance between the solvent entry position and the sample application zone, although this fact is often overlooked [32,34]. Small and repeatable values for the plate height require first of all that Z_0 is small and secondly maintained constant over a series of measurements. The effect of Z_0 on H_{obs} is indicated in Fig. 2 for a HPTLC silica layer under capillary flow conditions. A high mobile phase velocity at the start of chromatography (i.e., when the solvent front

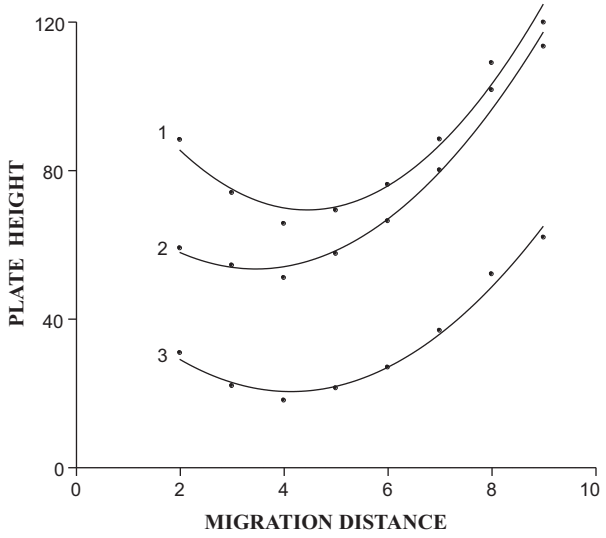

Fig. 1. Variation of H_{obs} (μm) at $R_F = 1$ as a function of the solvent front migration distance Z_f (cm) with $Z_0 = 5$ mm for a Whatman HPTLC HP-K silica gel layer and n-hexane as the mobile phase. Correction for contributions from σ^2_{SA} where made using (1) the dimensions of the sample zone prior to development; (2) the dimensions of the sample zone after migrating a short distance; and (3) using the rest diffusion value (Kaiser method).

reaches the sample application zone) and throughout the separation is critical for maintaining system efficiency. By minimizing Z_0 a higher and more favorable mobile phase velocity is achieved for the separation reflected in smaller plate height values. For capillary flow the mobile phase velocity is not constant and declines with the solvent front migration distance (see Section 2.2).

2.2. Capillary flow

Migration of the mobile phase through a particle bed by capillary flow facilitates the use of TLC as a portable and inexpensive separation system requiring the minimum of instrumentation. Capillary flow remains the popular operating mode in modern TLC but also serves to limit the separation performance and zone capacity that can be achieved. Capillary forces are insufficient to provide an optimum flow velocity for high performance layers, to provide a constant velocity over typical development distances, and to provide a useful velocity over distances long enough to obtain a high zone capacity [19–21,35–37]. In the absence of exchange of vapors with the gas phase in contact with the layer the position of the solvent front after some time t from the start of development for capillary flow is given by:

$$Z_f^2 = \kappa t \tag{6}$$

where κ is the velocity constant and related to the system properties by

$$\kappa = 2K_0 d_p \left(\frac{\gamma}{\eta}\right) \cos\theta \tag{7}$$

and K_0 is the permeability constant, d_p is the average particle diameter, γ is the surface tension of the mobile phase, η is the viscosity of the mobile phase, and θ is the contact angle for the mobile phase on the stationary phase (wetting angle). To achieve the highest possible mobile phase velocity the layer should be homogenously and tightly packed (maximize K_0); contain particles of a relatively large particle size, and of equal importance, of a narrow size distribution [the derivation of Eq. (7) assumes the particles are spherical whereas irregular particles are mainly used for the preparation of pre-coated layers]; the mobile phase should be selected to maximize the ratio of its surface tension to viscosity and not simply to optimize either solvent property alone [20], and the mobile phase should completely wet the stationary phase. Relevant typical properties of pre-coated silica gel layers are summarized in Table 2. A striking trend in the production of pre-coated conventional TLC plates in modern times has been the gradual reduction in the average particle size and their size distribution. Commercial pre-coated layers available to workers in the 1960 had an average particle size of about 20 μm and a size distribution of 10–60 μm. The same layers today have an average particle size of 10–12 μm and a size distribution of 5–20 μm, and perhaps better, since the average plate height values from recent years suggest an improvement in quality when compared with HPTLC layers, whose characteristic properties have been more stable over time. A decrease in mobile phase velocity by a factor of 1.5 is observed for a 10-fold increase in the particle size distribution [37]. The zone capacity and average plate height for conventional and high performance pre-coated layers from some manufacturers are now quite similar, reflecting tighter control of particle size distributions, although HPTLC separations are significantly faster [1,25,38].

The quadratic flow relationship, Eq. (6), for capillary flow is reflected in the maximum solvent front development distances in Table 2. As the mobile phase velocity declines at some distance from the solvent entry position the progress of the solvent front is so slow that sample zones are expanding due to diffusion as fast, or faster, than they are migrating apart. Allowing further time for the separation will not lead to improved separations at this point, and indeed, the separation performance is expected to decline as the separation time (solvent front migration distance) is increased. Eventually the mobile phase will stop moving completely when capillary forces are no longer sufficient to move the column of mobile phase contained within the layer. For any selected particle size there is an associated maximum solvent front migration distance that increases with the average particle size. For capillary flow the use of layers with a smaller particle size would only allow for an increase in performance for a shorter migration distance, and consequently, with a lower zone capacity [11,12,25].

The layer thickness for a fixed particle size affects the mobile phase velocity and thinner layers result in a higher mobile phase velocity and a small increase in performance and sample detection limits [39]. A reduction of the layer thickness from 0.2 to 0.1 mm for silica gel HPTLC layers affords a solvent-dependent increase of 1.1–2.5-fold in the mobile phase velocity. As shown in Fig. 3, the plate height minimum for the 0.1 mm and 0.2 mm HPTLC layers are similar with the most noticeable difference being the shift in the minimum value to a longer migration distance for the thinner layer in keeping with the higher mobile phase velocity. For applications

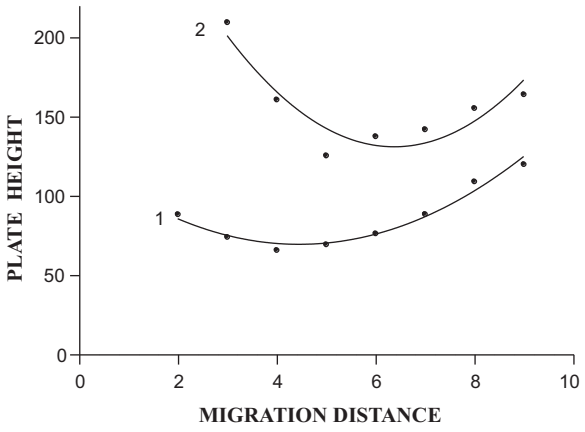

Fig. 2. Variation of H_{obs} (μm) with the solvent front migration distance for a HPTLC silica gel layer with n-hexane as the mobile phase and $Z_0 = 5$ mm (1) and 15 mm (2).

Table 2
Characteristic properties of pre-coated silica gel layers.

Parameter	TLC	HPTLC	UTLC
Plate size (cm)	20 × 20	10 × 10	6 × 3.6
Layer thickness (mm)	0.1–0.25	0.1 or 0.2	0.01
Particle size (μm)	10–12	4–6	Monolithic
Particle size range (μm)	5–20	4–8	
Maximum value for Z_f (cm)	7–15	3–7	1–3
Separation time (min)	30–200	3–20	1–5
Average plate height (μm)	35–75	23–25	
Typical application volume (spots) (μl)	1–5	0.1–0.5	0.01–0.1
Initial spot diameter (maximum) (mm)	3–6	1–1.5	0.5–1
Detection limits (reflectance)			
UV–vis (ng)	1–5	0.1–0.5	0.5
Fluorescence (pg)	50–100	5–10	5

involving repeated development of the layer, for example in automated multiple development, a considerable saving of time can be achieved by using thinner layers, but for single development applications the benefits are less significant and must be weighed against the lower sample capacity of the thinner layers. The compromise tends to favor the thinner layers but the more frequent use of 0.2 mm HPTLC layers would suggest that the modest enhancements in separation time, performance, and sample detectability are insufficient to make this the general choice for routine applications.

Silica gel is adequately wet by the majority of mobile phases used in thin-layer chromatography and the contact angle term in Eq. (7) can normally be omitted from calculations. This is not the case for chemically bonded stationary phases and mobile phases containing a significant volume fraction of water (typical reversed-phase separation conditions) [1,7,19]. Some characteristic properties of chemically bonded stationary phases used for thin-layer chromatography are summarized in Table 3. For the alkylsiloxane-bonded layers of various chain lengths (2 = ethyl; 8 = n-octyl; and 18 = n-octadecyl) the water compatible layers are defined by a lower bonded phase density and a larger average particle size to achieve an acceptable range of mobile phase velocities for reversed phase separations. Those layers with a higher bonded phase density and smaller particle size are intended for use with a high-organic content mobile phase only. Without adjusting the physical and chemical properties of the layers capillary forces would be too weak to generate a useful mobile phase velocity for aqueous mobile phases largely as a result of the poor contact angle on the stationary phase. This is not a problem for polar chemically bonded and cellulose stationary phases that are generally adequately wet by most common mobile phases, including water. For the alkylsiloxane-bonded phases the performance characteristics are closer to those associated with conventional silica gel layers but with longer separation times if the volume fraction of water is high.

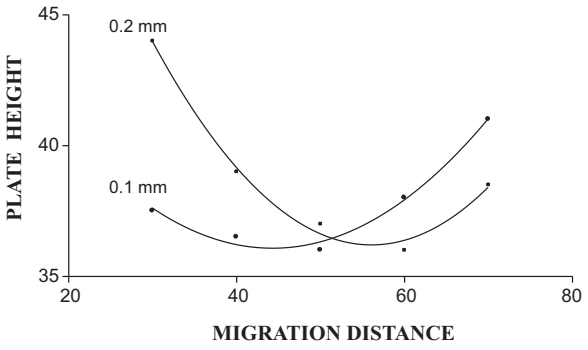

Fig. 3. Variation of H_{obs} (μm) at R_F = 0.5 with the solvent front migration distance (Z_f) for HPTLC silica gel layers of 0.1 and 0.2 mm thickness. General experimental conditions are given in [39].

As the mobile phase permeates the layer driven by capillary forces, interparticle channels of narrower diameter fill first leading to more rapid advancement of the mobile phase. Large intraparticle pores behind the solvent front fill more slowly, resulting in an increase in the thickness of the mobile phase layer. The bulk mobile phase velocity (saturated flow) through the region occupied by the sample zones is slower than the solvent front velocity. The bulk mobile phase velocity is more relevant for determining system properties. It can be determined by adding an unretained and visible or detectable compound to the sample (or neighboring track to the sample track), or alternatively is assumed to be about 0.8 times the observed unsaturated solvent front velocity [20,40–42]. The factor 0.8 is a reasonable approximation often used in simulation studies, but real experimental values may be slightly different.

Due to the presence of a vapor phase in contact with the layer the mobile phase velocity is dependent on the type of developing chamber and its saturation level [43]. Two opposing phenomena affect the mobile phase velocity. Vaporization of solvent from the wetted area of the layer, which depends on the vapor pressure of the solvent in the chamber, results in a reduction of the mobile phase velocity. Simultaneously, after insertion of the dry layer into the developing chamber, the layer progressively adsorbs mobile phase vapors slowly filling the pores of the unwetted layer ahead of the advancing solvent front. The apparent layer porosity diminishes and the solvent front velocity slowly increases with time. Sandwich chambers, which eliminate or minimize contact between the layer surface and the vapor phase, afford greater reproducibility of the mobile phase velocity between separations than obtained in classical large volume chambers. The choice of the developing chamber, volume of mobile phase, and the degree of saturation affect plate height measurements since they affect the mobile phase velocity. When comparing different data sets they should not be allowed to become uncontrolled variables.

2.3. Forced flow

It is now widely accepted that separation performance in thin-layer chromatography is primarily restricted by inadequacies of the mobile phase velocity created by capillary forces. The mobile phase velocity decreases with the migration distance of the solvent front and the maximum velocity at any position on the layer is less than the velocity required for optimum performance [23–26,44]. For typical separation conditions on HPTLC layers by capillary flow the mobile phase velocity is between 0.2 and 0.05 mm/s while the optimum velocity for these layers is about 0.5 mm/s [44]. Conceptually, an obvious solution to this problem is forced flow development in which a constant mobile phase velocity for the separation is achieved by sealing the open side of the layer and using an external device to supply the mobile phase to the layer at a constant and selectable flow. The open surface of the layer can be sealed using a

Table 3
Characteristic properties of precoated chemically bonded layers.

Manufacturer	Derivatizing reagent	Percent silanol groups reacted	Carbon loading (%)	Average particle size (μm)
Merck				
RP-2	Bifunctional	50		5–7
RP-8 W	Bifunctional	25	8.9	11–13
RP-8	Bifunctional	37		5–7
RP-18 W	Bifunctional	22	15.4	11–13
RP-18	Bifunctional	35		5–7
3-Aminopropyl	Trifunctional	50	5.8	5–7
3-Cyanopropyl	Bifunctional	27		5–7
Whatman				
KC-2	Monofunctional		4.5	10–14, or 20
KC-8	Monofunctional		8.5	10–14
KC-1	Trifunctional	16	12.5	10–14
Diphenyl	Difunctional		8.5	10–14
Macherey-Nagel				
Sil C18-100	Trifunctional	45		5–10
Sil C18-50	Trifunctional	30		5–10

flexible polymer or metal membrane [45–49], or optically flat rigid surface [50], under hydraulic pressure. The pressure required to seal the layer must exceed the inlet pressure required to overcome the flow resistance of the layer at the selected mobile phase velocity. Commercially available instruments operate at pressures up to 100 bar with some self-built chambers operating at over 200 bar. For a typical development length of 18 cm an overpressure <100 bar is suitable for operating layers with an average particle size >3 μm [23–25]. In studies of layer performance two general operating modes known as fully on-line and off-line are used. In the fully on-line mode the system is operated in a manner similar to column liquid chromatography with the layer considered as a flat column. The sample is introduced into the pressurized mobile phase using a loop injection valve, which then passes through a dispersion element (narrow trough along one side of the layer) to reshape the sample plug into a narrow band driven across the layer by the flow of mobile phase to a second dispersion element that collects the mobile phase and passes it into a flow through detector. In this mode the stationary phase can be equilibrated with the mobile phase prior to introduction of the sample. Several different operational strategies are possible in the off-line mode. Typically the sample (or samples) is applied to the dry layer as spots or bands, the separation is then achieved by development for a selected distance (a dispersion element is still required to reshape the mobile phase flow into a linear solvent front), and the sample zones are subsequently detected by scanning densitometry, or less commonly, by elution from the layer. In the forced flow mode the mobile phase velocity is no longer dependent on the contact angle between the mobile and stationary phases for reversed-phase chromatography. Also, the mobile phase velocity is constant throughout the separation and can be adjusted to different selected values.

When the mobile phase is forced through an initially dry layer of porous particles sealed to the external atmosphere the air displaced from the layer by the liquid usually results in the formation of a second solvent front (beta front) moving behind the solvent–air front (alpha front), which is often wavy in character [51,52]. During the liquid–air displacement process not all the air is displaced instantaneously escaping ahead of the solvent front. Some is displaced at a slower rate and must leave the sorbent by dissolution in the mobile phase or as microbubbles moving with the mobile phase. The solubility of the air in the mobile phase depends on the pressure used to seal the layer, and above some critical pressure, all of the gas will dissolve. The distance between the alpha and beta fronts is generally referred to as the disturbing zone. Sample zones moving in the disturbing zone or passed over by it are often distorted and cannot be used for plate height measurements. The disturbing zone can be eliminated or minimized by pre-development of the layer with a solvent in which the sample does not move so as to dislodge the trapped air from the layer prior to initiating the separation. Alternatively, a backpressure regulator can be used to increase the local pressure and thus the solubility of the air in the mobile phase.

Forced flow separations have been performed using pre-coated conventional and high performance layers and packed beds containing a binder as well as packed beds without a binder. The packed beds are prepared by spreading a slurry of the stationary phase (and binder, if used) in a rectangular trough of the desired depth, removing excess solvent by evaporation, and sealing the bed on its open side by a pressurized polymer or metal membrane. For slurry packed beds prepared from 3 μm spherical or 5 μm irregular silica particles Flodberg and Roeraade reported minimum plate heights of about 17 and 34 μm and an optimum velocity of 0.61 and 0.25 mm/s, respectively, using forced flow development [53]. Mincsovics et al. [48] obtained plate heights as small as 37 μm on pre-coated high performance silica layers using forced flow development in the fully on-line mode.

A common problem for plate height measurements using the overpressured development chamber is the external volumes associated with connecting tubing, dispersion elements, etc., which are larger than desirable and not easily reduced [48,54]. In addition, when samples are applied as spots or bands to the dry layer a correction is required for the finite size of the sample application zones, which are of a significant size with respect to the dimensions of the separated zones, but no agreement exists as to how this should be achieved (see discussion in Section 2.1).

Forced flow was used in a systematic study of the kinetic properties or pre-coated conventional and high performance silica gel layers employing an overpressured developing chamber modified to minimize external volumes and using peak shape modeling to extract the true band broadening characteristics for the layers [44,54–56]. Typical results are summarized in Table 4 for the different layers (individual values for pre-coated layers manufactured by Merck, Whatman, and Macherey-Nagel can be found in the cited references). Unlike columns, pre-coated layers have a heterogeneous composition containing a binder as well as sorbent particles, and also possibly a fluorescent indicator for visualization, such as manganese-doped zinc silicate particles, or similar materials. Typical binders include gypsum or salts of poly(acrylic acid), in amounts of 0.1–10% (w/w), to impart the desired mechanical strength and durability to the layers to facilitate convenient handling in the laboratory with minimal risk of damaging the layer. General models used to interpret the kinetic properties of layers using fully on-line forced flow methods are identical to those developed for columns

Table 4
Characteristic kinetic properties of pre-coated silica gel layers determined by fully on-line forced flow development [44,54–56].

Parameter	Layers	
	High performance	Conventional
Porosity		
Total	0.65–0.70	0.65–0.75
Interparticle	0.35–0.45	0.35–0.45
Intraparticle	0.28	0.28
Flow resistance parameter (θ)	875–1500	600–1200
Apparent particle size (μm)	5–7	8–10
Minimum plate height (μm)	22–25	35–45
Optimum mobile phase velocity (mm/s)	0.3–0.5	0.2–0.5
Minimum reduced plate height	3.5–4.5	3.5–4.5
Optimum reduced mobile phase velocity	0.7–1.0	0.6–1.2
van Deemter equation [$H = A + (B/u_e) + Cu_e$] coefficients[a]		
A ($\times 10^4$) cm	2.5–4	15–27
B ($\times 10^6$) cm^2/s	60–80	40–80
C ($\times 10^3$) s	10–20	15–70
Knox equation [$h = Av^{1/3} + (B/v) + Cv$] coefficients[a]		
Flow anisotropy (A)	0.4–0.8	1.7–2.8
Axial diffusion (B)	1.2–1.6	1.2–2.0
Resistance to mass transfer (C)	1.4–2.4	0.70–0.85
Separation impedance (E) $\times 10^3$	10–20	11–13

[a] u_e = interparticle mobile phase velocity; v = reduced mobile phase velocity; and h, reduced plate height defined in Ref. [44].

and do not consider specific contributions from the binder and indicator except as a potential explanation for why the properties of layers deviate from those of columns. The pre-coated layers have similar porosity with average values for the total porosity of 0.69, interparticle porosity 0.42, and intraparticle porosity 0.27 [54]. These results suggest that the packing density for columns and layers are quite similar (comparable interparticle porosity values). The total porosity and intraparticle porosity of layers, however, are significantly smaller than those for slurry packed columns suggesting that a significant amount of the binder used to stabilize the layers is contained within the porous particles. Evaluation of the pore size distribution of the pre-coated layers supports the hypothesis that a significant fraction of the binder is contained within the pores and not merely blocking the pore entrances [55]. The flow resistance parameter for conventional pre-coated layers is less than values for the high performance pre-coated layers, Table 4. This suggests that the permeability of conventional pre-coated layers provide a more optimum packing density, independent of particle size differences. The difference in flow resistance may result from the deleterious effect of the presence in the fine particle layers of greater amounts of particles of less than average size because the average particle size is smaller than for the larger particles used to prepare pre-coated conventional layers. The apparent average particle size for the high performance layers, 6.3 μm, is significantly larger than the nominal value stated by the manufacturer, 5 μm, while for the conventional layers the apparent average particle size, 9 μm, is considerably smaller than would be expected from the nominal particle size range of 10–15 μm indicated by the manufacturers of the pre-coated layers. Since the apparent particle size is calculated from the permeability of the layers, the differences observed are probably due to features of the particle size distribution, as discussed for the flow resistance parameter. On the other hand, given the heterogeneous nature of the layers and the difference in average particle sizes typically obtained with different measurement techniques, there is reasonable agreement between the experimentally observed results and the nominal layer properties. The influence of the mobile phase velocity (or reduced velocity) on zone broadening can be quantitatively evaluated by fitting experimental results for the plate height (or reduced plate height) to the van Deemter and Knox equations [44]. Typical results for a van Deemter

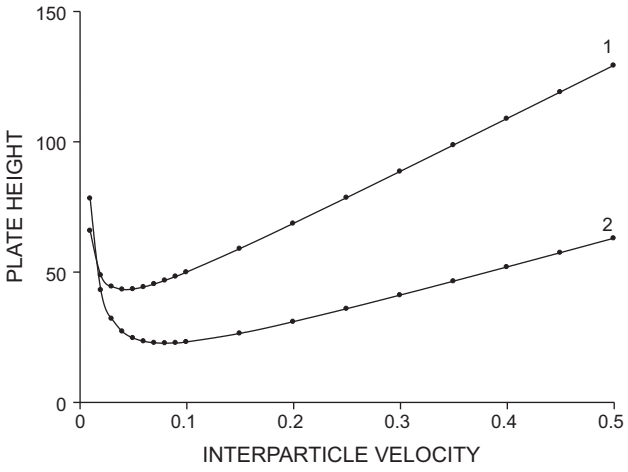

Fig. 4. Variation of the plate height H (μm) with the interparticle mobile phase velocity u (mm/s), van Deemter plot, for Whatman pre-coated conventional (1) and high performance (2) silica gel layers determined by fully on-line forced flow development. Experimental details in Ref. [44].

plot for conventional and high performance pre-coated layers are shown in Fig. 4. The minimum plate height for the high performance pre-coated layers at 22–25 μm is considerably better than for the conventional layers at 35–45 μm and occurs at a similar optimum interparticle mobile phase velocity of about 0.1 mm/s. The minimum plate height values are about 1.5–3-fold larger than for an ideal column ($H \approx 2d_p$) with an optimum linear velocity 2–4-fold lower. The values for the high performance pre-coated layers are closer to the column values than those for the conventional layers.

A better impression of the difference in properties for the pre-coated layers can be gathered from the plot of the reduced plate height against the reduced velocity in Fig. 5. The minimum reduced plate height for conventional pre-coated layers, 4.2–7.5, are generally larger than for the high performance pre-coated layers, 3.6–4.2, both of which are significantly larger than for an ideal column, 1.5–3, and observed at a lower optimum reduced velocity of about 0.8 compared with 3–5 for an ideal column. The reasons for the differences in kinetic properties for the pre-coated layers can be deduced from the coefficients of the Knox equation (Table 4)

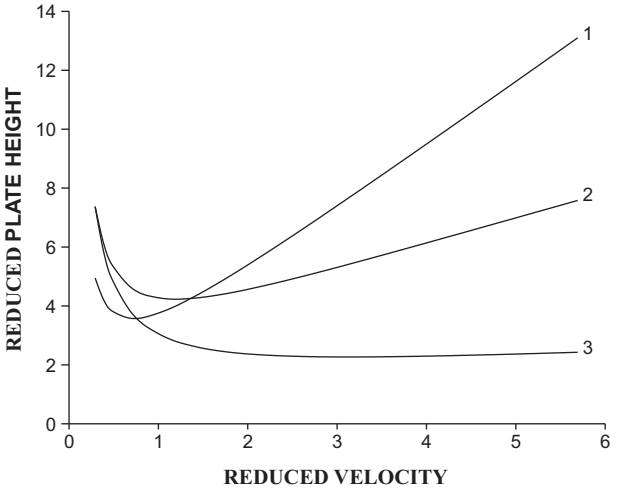

Fig. 5. Variation of the reduced plate height with the reduced velocity (Knox plot) for Merck pre-coated silica gel layers determined by fully on-line forced flow development. Identification: 1, high performance layer; 2, conventional layer; and 3, an ideal column ($A = 1$, $B = 2$, and $C = 0.05$). Experimental details in Ref. [44].

Table 5
Typical properties of reversed-phase octadecylsiloxane-bonded silica layers used in pressurized planar electrochromatography (Merck).

Property	TLC RP-18	HPTLC RP-18	LiChrospher RP-18W
Particle shape	Irregular	Irregular	Spherical
Average particle diameter (μm)	10–12	5–6	6–8
Particle size distribution (μm)	5–20	4–8	
Layer thickness (mm)	0.25	0.2	0.2
Coverage density (μmol/m^2)	2.6	2.6	0.5

[44,56]. The *A* term characterizes the flow anisotropy within the streaming portion of the mobile phase and is related to the uniformity and packing density of the layer. The *A* coefficients for the high performance pre-coated layers are similar or smaller than those for a typical column ($A \approx 0.5$–1.0) indicating that the layers are homogeneously packed. Conventional pre-coated layers are not as homogeneously packed, perhaps because of the greater difficulty of coating layers with particles of a larger particle size or because the particle size ranges of conventional TLC sorbents are wider than desirable.

The *B* term characterizes the contribution of longitudinal diffusion to the plate height and should be similar in value for layers of different types, as observed. The *C* term is a measure of the resistance to mass transfer between the solvated stationary phase and the streaming mobile phase. Typical values for columns, 0.05–0.70 [44], are on average up to an order of magnitude smaller than those observed for the pre-coated layers. The larger values of the *C* term for the layers may be a result of restricted diffusion within the porous particles or different rates of sorption for the silica gel surface and that portion of the silica gel surface coated with binder. For forced flow separations the large *C* term contributes to both the higher minimum plate height and the lower optimum mobile phase velocity compared with typical columns. Improvements in layer properties aimed by reducing the contribution to zone broadening from resistance to mass transfer would be useful for force flow applications but may make little difference to capillary flow systems where the average plate height for high performance pre-coated layers is dominated by contributions from longitudinal diffusion [32,56].

2.4. Pressurized planar electrochromatography

Electroosmosis is used to drive the mobile phase flow through the layer in pressurized planar electrochromatography and requires an aqueous buffer as a component of the mobile phase [23,57–62]. An electric field applied across the layer is used to control the mobile phase velocity which is independent of the solvent front migration distance. All practical applications employ chemically bonded pre-coated layers (Table 5) or polymeric monolithic films with ionic functional groups for separations by reversed-phase chromatography. The mobile phase velocity depends on the physicochemical properties of the mobile phase and the mobile–stationary phase interface and is generally higher for silica-based chemically bonded phases with a lower bonding density and is independent of the particle size. Problems associated with either excess heating of the layer or accumulation of liquid on the layer surface can be avoided by applying pressure to the layer, in a manner that allows heat to flow between the layer and the pressurizing mechanism, typically a special metal block and hydraulic press with some mechanism, such as circulating liquid, to control temperature [57]. In an open system liquid accumulates on the surface of the layer causing distortion of sample bands, and if too much heat is generated, the separation is terminated due to drying of the layer. Overpressure and temperature control are required to control these processes.

There are specific problems associated with sample application since the layer must be completely impregnated with the mobile phase at the start of the separation while samples are typically applied to the dry layer [57,63]. In addition, sample zones applied to the layer are generally too large to obtain optimum performance and cited plate height measurements are usually calculated by subtraction of the variance due to the sample application zone. Typical sample application volumes are restricted to about 10–20 nL for spots or bands to minimize the initial size of the starting zone before wetting the layer. A number of operational steps need to be completed quickly and reproducibly requiring some skill prior to inserting the layer into the developing chamber.

Plate height measurements have been made in the development mode and depend on the migration distance. The plate height generally declines with increasing migration distance reaching a plateau or shallow region between about 35 and 75 mm [57,61,63,64]. The lowest observed plate heights for the plateau region fall into the range 10–15 μm corresponding to $H \approx 2d_p$ for high performance pre-coated octadecylsiloxane-bonded silica layers. Larger plate heights are observed for pre-coated conventional layers due to the larger average particle size and broader particle size distribution. A significant difference for the pre-coated layers is the steeper ascending portion of the plate height against mobile phase velocity (or flow rate) curve at higher flow rates for conventional pre-coated layers compared with the almost flat curve for high performance pre-coated layers over the mobile phase velocity range 0.3–1.2 mm/s. The results from pressurized planar electrochromatography are quite promising for reversed-phase separations although the instrumentation is at an early stage of development and no commercial sources currently exist.

3. Plate height models for stabilized particle layers

Major contributions to the kinetic theory of planar chromatography were made by Giddings and co-workers [65,66], de Ligny and Remijnsee [67,68], Kaiser [6], Guiochon, Siouffi and co-workers [35,40,69–72], and Belenkii and co-workers [37,73] in the period from 1960 to the early 1980s. These studies and others were reviewed in Geiss's classical text on the fundamental aspects of thin-layer chromatography [20] and more recently by Spangenberg et al. [19]. In recent years further advances in theory have been few, although considerable progress has been made in instrumentation, measurement techniques, and in consolidating existing knowledge [19–25]. For this reason we have limited our discussion in this section to a few points that offer some insight into the relationship between layer properties and zone broadening and what these suggest for improving the performance of thin-layer separations using stabilized particle layers.

General models used to explain the relationship between the plate height and layer structure for capillary flow separations are based on models developed for columns adapted to accommodate the specific experimental conditions pertinent to thin-layer chromatography. The quality of available experimental data and an inability to control the experimental parameters, as can be done in a closed column system, was influential in dictating the approaches used as well as a reasonably mature theory being available for column chromatography [20,21]. The model proposed by Guiochon and Siouffi is intuitively most appealing and is based on refinements suggested in earlier models [35,40,69–72]. The assumptions made are:

(1) The layer has the same properties as a typical column bed.
(2) The mobile phase velocity is constant at all positions within the layer at a given time but decreases with increasing time.

(3) The local plate height can be fully described by the Knox equation.
(4) The composition of the mobile phase is constant throughout the layer.
(5) The zone focusing effect when the sample application zone is first contacted by the mobile phase is negligible.

With a few further simplifying assumptions, these authors arrived at the general model, Eq. (8) for the observed plate height:

$$H_{obs} = [a/(Z_f - Z_0)][(Z_f^{2/3} - Z_0^{2/3})] + b[/(Z_f + Z_0)] + [c//(Z_f - Z_0)]\log\left(\frac{Z_f}{Z_0}\right) \quad (8)$$

where $a = 1.5A(d_p{}^4\kappa_B/2D_M)^{1/3}$; $b = BD_M/\kappa_B$; $c = C\kappa_B d_p{}^2/2D_M$; A, B and C are the coefficients of the Knox equation (see Table 4); κ_B is the apparent mobile phase velocity constant for bulk flow (assumed equal to 0.8κ); and D_M is the solute diffusion coefficient in the mobile phase. In practice, the contribution of longitudinal diffusion to the plate height may be more complex than represented by the b coefficient in Eq. (8). The contribution from molecular diffusion in the stationary phase may be of the same order of magnitude as its contribution from the mobile phase in some circumstances, in which case, the plate height is no longer independent of the residence time of the solute in the stationary phase and the contribution from molecular diffusion is more faithfully represented by Eq. (9):

$$BD_M = 2\left[\lambda_M D_M + \left\{\frac{(1 - R_F)}{R_F}\right\}\lambda_S D_S\right] \quad (9)$$

where λ is the tortuosity factor and the subscripts S and M refer to the stationary and mobile phases, respectively. The difficulty with Eq. (9) is that the exact form of the contribution from diffusion in the stationary phase is unknown. Two limiting conditions can be defined. The stationary phase contribution to longitudinal diffusion is zero ($\lambda_S D_S = 0$), in which case the b coefficient reverts to BD_M/κ_B. Alternatively, the contribution from diffusion in the mobile and stationary phases are assumed to be equal ($\lambda_M D_M = \lambda_S D_S$) and the b coefficient is dependent on the R_F value ($b = BD_M/R_F\kappa_B$). Simulations of the relationship between the plate height and solvent front migration distance for pre-coated conventional and high performance layers based on Eq. (8) are shown in Figs. 6 and 7, respectively [32]. The simulations demonstrates that the contributions from resistance to mass transfer to the plate height (H_c) is negligible for pre-coated conventional and high performance layers because the range of mobile phase velocities generated by capillary forces are too slow. This is different to forced flow conditions where resistance to mass transfer limits the performance of stabilized particle layers at moderate to high flow rates (see Section 2.3). The dominant contribution to the plate height is longitudinal diffusion, the b term in Eq. (8), which increases as the mobile phase velocity decreases. This is the only term of significance for separations on pre-coated high performance layers. Since the observed plate height is dominated by the b term, there is only a weak dependence on the average particle size but a strong dependence on the solvent front migration distance. The opposite is true for forced flow conditions where the mobile phase velocity is both higher and constant as a function of the solvent front migration distance. Increasing Z_0 for capillary flow systems does not change the shape of the plate height relationship but leads to a series of near parallel lines shifted to higher plate height values. The a term (flow anisotropy) makes a significant contribution to the observed plate height for pre-coated conventional layers but is negligible for pre-coated high performance layers. The contribution of flow anisotropy to the observed plate height declines slightly with increasing solvent front migration distance and is more important at the start of the separation when the mobile phase velocity is at its highest.

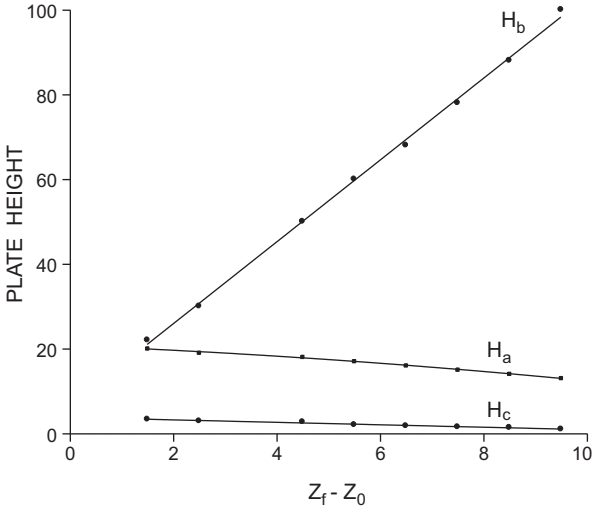

Fig. 6. Simulation of the contribution of flow anisotropy (H_a), longitudinal diffusion (H_b), and resistance to mass transfer (H_c) to the observed plate height (μm) as a function of the migration distance ($Z_f - Z_0$) in cm based on Eq. (8) for a pre-coated silica gel TLC layer using typical parameters given in Table 4.

A comparison of experimental data for the observed plate height with values simulated by Eq. (8) reveals obvious discrepancies [21,32]. For the pre-coated high performance layers, the simulated values are always larger than the experimental values and this difference increases for longer solvent front migration distances. The agreement is better for pre-coated conventional layers, but is still no better than fair. In both cases the simulations do not imitate the upward curvature of the experimental plots at short solvent front migration distances (i.e. compare Figs. 1 and 7). Thus Eq. (8) fails to fully describe the plate height relationship with solvent front migration distance for capillary flow and needs to be used advisedly.

The approach proposed by Belenkii et al. [73] is based on the definition of an optimum plate length (L_{opt}) corresponding to an

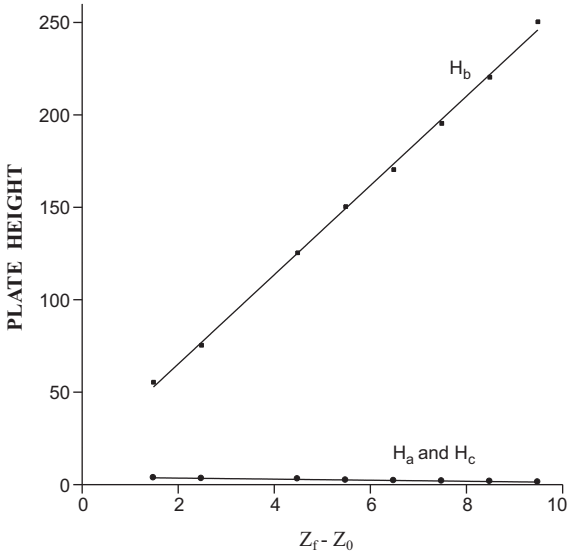

Fig. 7. Simulation of the contribution of flow anisotropy (H_a), longitudinal diffusion (H_b), and resistance to mass transfer (H_c) to the observed plate height (μm) as a function of the migration distance ($Z_f - Z_0$) in cm based on Eq. (8) for a pre-coated silica gel HPTLC layer using typical parameters given in Table 4.

optimum reduced velocity (ν_{opt}). The plate height is related to the mobile phase velocity using the Knox equation, except that since the reduced velocity is not constant for capillary flow the instantaneous reduced plate height depends on the migration distance and is related to the solvent front migration distance. For higher values of L, the reduced plate height, h, increases, passes through a minimum h_{min}, and then begins to increase sharply. Optimization of a separation is obtained by the choice of a layer with $L = L_{opt}$ corresponding to ν nearly equal to ν_{opt} at the end of the separation. For this condition $h = h_{min}$ for the entire length of the plate (solvent front migration distance). With these assumptions we obtain the model:

$$h = [1.5a\nu^{1/3}(1-\delta^{2/3})/(1-\delta)] + [0.5b(1/\nu)(1+\delta)] + [c\nu\{\ln(1/\delta)/(1-\delta)\}] \quad (10)$$

The three terms in square brackets (in order) represent the contributions from flow anisotropy, longitudinal diffusion, and resistance to mass transfer. The variable $\delta = \nu/\nu_0$ is the ratio of the reduced velocity at the end of the separation, ν, to the reduced velocity at the start of the separation, ν_0. In column chromatography ν is constant while it varies in thin-layer chromatography and the variable δ is a correction for this difference in the Knox equation. It was shown that for small values of δ the reduced plate height could be described by

$$h = 1.5a\nu^{1/3} + 0.5b(1/\nu) + c\nu\ln[(1/\delta)/(1-\delta)] \quad (11)$$

Optimum separation conditions are achieved by choosing a plate of an optimum length, $L_{opt} = 5\alpha^2 {d_p}^2/D_M$ where $\alpha = 0.5K_o d_p \gamma \cos\theta/L\eta$ with the variables defined for Eq. (3). Belenkii et al. proposed that only two separation systems were useful for thin layer chromatography. For low molecular weight compounds a development length of 10 cm and layers coated with 10 μm particles was desirable. For high molecular weight compounds a development length of 5 cm and layers coated with 5 μm particles was preferred. These results are broadly in agreement with experimental results provided that both layers have a narrow particle size distribution. The poorer performance of pre-coated conventional layers is likely due to their wider particle size distribution compared with the above predictions.

4. Particle membranes

Particle-loaded membranes consist of spherical silica gel or silica-based chemically bonded particles of about 8 μm particle diameter enmeshed in a network of poly(tetrafluoroethylene) microfibrils forming a strong porous sheet with a ratio of sorbent-to-microfibrils of about 9:1 by weight [74–77]. Particle-embedded membranes contain silica or chemically bonded particles of 10–30 μm average particle diameter, but with a narrow size range, woven into a glass fiber-supporting matrix [78]. The particle-embedded membranes are used as the separation medium in the Toxi-Lab system for toxicological drug screening by thin-layer chromatography [79]. Both particle-loaded and particle-embedded membranes are used in the form of disks for solid-phase extraction [80].

The separation performance of particle-loaded membranes for capillary flow conditions are similar to pre-coated conventional TLC layers as demonstrated in a number of applications [76]. The characteristic kinetic properties of particle-loaded and particle-embedded membranes determined under forced flow conditions and are summarized in Table 6 [44,54–56,77,78]. These values can be compared directly with those for the stabilized particle layers indicated in Table 4. The general models used for calculation of kinetic parameters were developed for packed particle beds

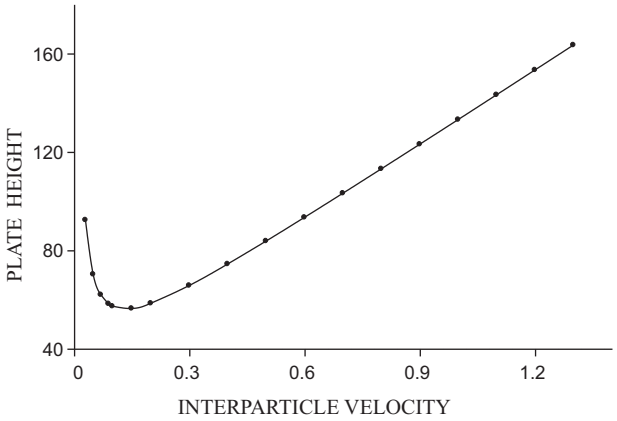

Fig. 8. Plot of the observed plate height as a function of the interparticle mobile phase velocity for an octadecylsiloxane-bonded silica particle-loaded membrane. Measurements were made by fully on-line forced flow development. Experimental details in Ref. [77].

(columns) and do not explicitly consider the role of the support matrix. The results can be viewed as approximate or representing the apparent properties of the membranes with a particle bed as reference. The total porosity and interparticle porosity of the particle-loaded membranes suggest a lower packing density compared with stabilized particle layers. The binder in this material is a network of inert microfibrils which have to be accommodated in the interparticle space. In addition, some of the microfibrils must be contained in the pores of the particles or block the pore entrances. For the octadecylsiloxane-bonded particles it is likely that some of the pores are blocked by the reagent used to modify the surface (the chemically bonded phases have a high bonding density [78]).

For the particle-embedded membranes the intraparticle porosity is very small and blocking of the pores by the reagent used to modify the surface must be nearly complete. The apparent particle size for the particle-loaded membranes is in good agreement with the manufacturer's claims. The high specific permeability of the particle-embedded membranes indicates favorable flow characteristics. The glass fiber medium is a heterogeneous mixture of glass fibers with embedded sorbent particles with an apparent particle diameter of 15.3 μm [78]. This is in agreement with the plate height measurements for the particle-embedded membranes which exhibit no minimum over the mobile phase velocity range 0.2–3 mm/s. The van Deemter plot is essentially linear indicating that the plate height is dominated by resistance to mass transfer typical of relatively large porous particles. The van Deemter plots for the particle-loaded membranes are similar to those observed for stabilized particle layers (Fig. 8), although the minimum plate height is significantly larger and occurs at a lower optimum mobile phase velocity [77].

The values for the van Deemter and Knox coefficients indicate that for the particle-loaded membranes the contributions from flow anisotropy, mainly, and resistance to mass transfer are larger than those for stabilized particle layers. This feature is presumably a property of the microfibrils used as binder leading to a lower packing density as well as restricting access to the pore volume. Alternatively, the web of microfibrils may succeed in trapping portions of the mobile phase in the interparticle space, increasing the volume of stagnant mobile phase, compared with the volume of streaming mobile phase, leading to an increase in the resistance to mass transfer term. The general conclusion is that there are no specific advantages to the particle membrane format for thin-layer chromatography; they have properties similar to pre-coated conventional layers when capillary flow conditions are used for

Table 6
Characteristic kinetic properties of particle membranes determined by fully on-line forced flow development [44,54–56,77,78].

Parameter	Particle-loaded membranes		Particle-embedded membranes
	Silica gel	ODS[a]	ODS[a]
Porosity			
Total	0.59–0.61	0.52–0.54	0.51
Interparticle	0.33	0.37	0.47
Intraparticle	0.26–0.28	0.15	0.04
Flow resistance parameter (θ)	1300	1000–1200	900–1000
Apparent particle size (μm)	7.3	7.7	15.3
Minimum plate height (μm)	81.2	56	
Optimum mobile phase velocity (mm/s)	0.34	0.13	
Minimum reduced plate height	8.3	7	
Optimum reduced mobile phase velocity	0.8	0.75	
van Deemter equation [$H = A + (B/u_e) + Cu_e$] coefficients			
A ($\times 10^4$) cm	54.6	28.9	
B ($\times 10^6$) cm^2/s	45.1	18.1	
C ($\times 10^3$) s	39.4	102	
Knox equation [$h = Av^{1/3} + (B/v) + Cv$] coefficients			
Flow anisotropy (A)	5.73	3.75	
Axial diffusion (B)	1.53	1.72	
Resistance to mass transfer (C)	1.55	1.54	
Separation impedance (E) $\times 10^3$	57	52	

[a] ODS, octadecylsiloxane-bonded silica particles.

separations and significantly poorer performance using forced flow conditions.

5. Ultra thin-layer chromatography

5.1. Monolithic films

Monolithic films of silica gel [13,81,82] or organic polymers [58,83,84] are thin, continuous biporous structures cast on a supporting surface. These structures do not require a binder to form a stable layer. Silica monoliths have been optimized for the separation of small molecules and polymer monoliths for life science applications. The characteristic properties of silica monoliths are summarized in Table 2.

Silica monoliths are formed by hydrolysis and condensation of alkylsiloxanes and a mixture of porogens on a glass plate [13,81]. The mechanically stable layers are about 10 μm thick with mesopores of 3–4 nm to optimize retention (specific surface area of about 350 m^2/g) and flow through macropores of 1–2 μm to minimize flow resistance. Compared with stabilized particle layers (Section 2.2) they provide faster separations, improved mass detection limits, and lower solvent consumption at the expense of lower resolution and increased handling complexity. Sample application is problematic due to the low sample volumes and small application zones needed for optimized separations, a direct result of the thin film and short migration distances (see Table 2). The significant transmission of light by monolithic films together with the small zone dimensions and short migration distances adds to the problems of quantitative measurements by scanning densitometry. Samples applied using standard laboratory equipment for high performance thin-layer chromatography indicate rather poor performance [13,81,82]. A zone capacity of only 5–6 with plate heights of 80–100 μm for low molecular weight compounds with R_F values between 0.1 and 0.5 were obtained [81]. There is no indication of how the plate height measurements were made or if corrections for the size of the sample application zone were made. Sample volumes of 5–200 nL applied by spotting or aerosol applicators are about 1 mm wide in the direction of development while separated zones are only about 1.5 mm. Thus the initial spot size is much too large for efficient separations. Morlock and co-workers [85] have shown that a better match between the operational requirements of monolithic films, and also nanostructured films (Section 5.3), can be achieved by replacing equipment optimized for high performance thin-layer chromatography with modified office computer peripherals, such as inkjet printers for sample application and flat bed scanners for chromatogram recording. Using a modified inkjet printer they were able to apply spots of 0.45–0.87 mm diameter (sample volumes of about 1–4 nL) and 3 mm bands of 0.2–0.6 mm height (sample volumes of about 3–10 nL). Reasonable mass detection limits were obtained using flat bed scanners, in the 10 s of nanogram range for strongly absorbing dyes in the visible region of the spectrum (but these values represent quite poor concentration detection limits). The authors do not report any plate height measurements. An emerging use of monolithic layers is in mass spectrometry where an increase in mass detection limits of an order or magnitude or more have been obtained using matrix-assisted laser desorption ionization (MALDI) [86,87] and surface desorption electrospray ionization techniques [88] compared with high performance stabilized particle layers. These improvements result mainly from a reduction in the layer thickness rather than an increase in separation performance.

Porous polymer monoliths are simply prepared in a thin film format using photoinitiated polymerization in a simple mold consisting of two glass plates, one of which provides the support structure for the monolithic film, separated with Teflon strips defining the film thickness [58,83,84]. Monolithic films 50–200 μm thick were prepared from poly(butylmethacyrlate-*co*-ethylene dimethacylate) monomers with other functional groups introduced by grafting to provide different retention mechanisms or a charged surface for pressurized planar electrochromatography (Section 2.4) [58]. There are no details of the kinetic performance of these layers or of their pore structure. They were used for the separation of simple protein, peptide, and dye mixtures by one- and two-dimensional thin-layer chromatography with capillary flow (except for [58]). Different mass spectrometric techniques were generally used for detection.

5.2. Electrospun nanofibrous layers

Electrospinning is a simple approach to producing polymeric fibers with nanometer diameters in lengths up to several meters. The motion of an electrically charged jet is used to deposit the fibers on a metal substrate as a mat of controllable thickness (Fig. 9). Materials evaluated for thin-layer chromatography include poly(acrylonitrile) fibers [89] and glassy carbon fibers prepared in situ by pyrolysis of a crosslinked poly(acrylonitrile) preformed

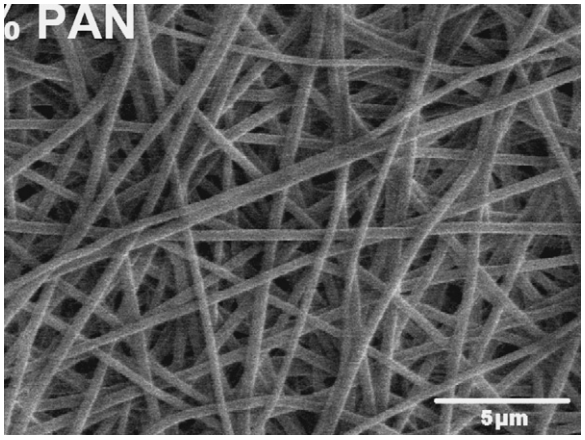

Fig. 9. Scanning electron microscopic image of an electrospun fiber layer prepared from poly(acrylonitrile) (10% polymer solution). Reproduced from Ref. [89] with permission.

layer [90]. The layers are sufficiently stable for general use without the need for a binder and were prepared in sizes from 2 to 3 cm wide and 6 cm long for separations. The poly(acrylonitrile) layers were made from fibers with 400 nm diameters and are 25 μm thick. The glassy carbon layers consist of fibers of 200–350 nm diameter and a thicknesses of 13–16 μm. Sample solutions of 50 nL were spotted by syringe to give starting zones of about 0.25–0.50 mm, and after development by capillary flow, separated zones <2.5 mm in diameter. Mobile phase velocity constants were generally higher than observed for high performance thin-layer plates. A remarkable feature of these layers is that for many compounds band broadening is minimal and constant plate height values <10 μm over development distances of 1–6 cm were observed. This performance is much higher than for stabilized particle layers using capillary flow (or forced flow). Since general expectations from molecular diffusion are not observed some focusing mechanism must be operative either during the separation or as the thin films dry out when removed from the mobile phase. Also, the structure of the layers is unique and there is no current theory to explain band broadening. Practical problems effecting performance are the low sample capacity of the layers, achieving sufficiently small sample application zone dimensions, the low specific surface areas of the fibers [21.7 m^2/g for the poly(acrylonitrile) fibers], weak and less selective interactions with organic polymers compared with inorganic oxides, and in the case of carbon layers, additional difficulties in optical detection of separated zones. The initial results are interesting and given the longer migration lengths possible compared with other ultra thin-layer formats the possibility of both high performance and high zone capacity should be easier to realize.

5.3. Nanostructured films

Nanostructured thin films can be prepared by the vapor phase deposition of electrothermally generated silicon dioxide using the glancing-angle deposition (GLAD) technique [14,91]. Computer-controlled biaxial substrate motion with deposition-rate feedback facilitates the growth of nano-columns with engineered shapes, such as helices, square spirals, vertical posts, and chevrons (zig-zag), as well as facilitating the introduction of macropores. Variation of the experimental conditions allows control of the film thickness. Isotropic vertical posts (diameter ≈ 200 nm, spacing ≈ 200 nm, and height 4.5 μm) and anisotropic chevrons (each segment of the zig-zag 0.5 μm and a height 5 μm) on one inch square glass substrates have been used most commonly for separations. The vertical posts were shown to collapse at the solvent evaporation stage limiting their reusability. Channel-like structures within the anisotropic films provide preferential mobile phase flow directions at an angle to the solvent front with separations occurring in diagonal tracks. This requires image analysis and specific software for recording separations. Plate heights of about 10–30 μm depending on the film morphology for solvent development distances <1.5 cm were observed. Samples applied as 10–25 nL spots by syringe had zone dimensions of a similar size to the migrated zones. At this early stage in the development of nanostructured films the reported conditions for film formation and film morphology are not necessarily optimal and so far no theoretical models are available to guide the design of these films. The nanostructured films share the common problems of the separation media for ultra thin-layer chromatography: thin films have a low sample capacity; lack of a practical method to apply sample zones with dimensions that are negligible (or at least small) with respect to the size of separated zones; difficulty in recording chromatograms by scanning densitometry; and greater experimental difficulty in controlling conditions for development. As discussed in Section 5.1, the use of modified office computer peripherals has shown some promise with respect to solving the instrumental difficulties [85].

6. Conclusions

Thin-layer chromatography is not a static subject and continues to evolve to meet different needs of its two major user groups. The first and less demanding group considers thin-layer chromatography as a fast and flexible laboratory technique for the separation of simple mixtures requiring simple and inexpensive equipment. For this group there is little that can be done to advance the performance of stabilized particle layers as long as capillary forces are used to provide the flow of mobile phase through the layers. Performance is defined by the inadequacies of this flow mechanism. Small improvements are possible by reducing the particle size distribution to increase the permeability of the layers and modestly reducing the layer thickness to increase the mobile phase velocity without significantly affecting sample capacity and ease of use. It is possible that spherical particles might also provide a modest improvement in performance compared with irregular particles commonly used. A question mark remains concerning the influence of the binder on layer performance, but this is probably not great for capillary flow systems and is of greater interest for forced flow methods.

For the second user group interested in expanding the scope of thin-layer chromatography to tackle more complex samples achieving higher performance will mean abandoning the use of capillary flow methods and exploring forced flow and pressurized planar electrochromatography with stabilized particle layers. This is the only sensible approach to tackle the dual problems of capillary flow: an inadequate mobile phase velocity for obtaining efficient separations and the dependence of the mobile phase velocity on the solvent front migration distance. Of the two approaches, forced flow can be considered mature, in that it has been in use for some time, but it is only recently that reliable and user friendly instrumentation has become available with suitable operational specifications for achieving high performance [23–25]. A problem still remains in achieving the necessary reduction in the external volume of instruments for use in the fully on-line mode. Current systems are capable of supporting a reduction in the particle size of stabilized particle layers to about 3 μm with a development length of 18 cm. This should allow optimum plate heights <10 μm to be obtained. Studies on superficially porous particles would be of interest as a mechanism to increase separation speed and performance by reducing the intrinsic plate height and facilitating the more economic use of pressure (similar to current developments in

column chromatography). By comparison pressurized planar electrochromatography is a promising technique but still in its infancy with much yet to be done to establish this method outside of the few research laboratories that have invested in the development of the technique. There are major problems to be resolved in the management of heat flow within the system for conditions suitable for high performance. The beacon for persistence is that this technique completely decouples the relationship between the mobile phase velocity and particle size that ultimately sets the performance limit for forced flow development. Since the efficiency of stabilized particle layers is scaled to the particle size then small particle layers with high flow rates could be used in pressurized planar electrochromatography yielding fast and efficient separations. This remains the hope but is not contemporary practice at this time. The normal phase separation mode is favored in planar chromatography. So far pressurized planar electrochromatography has been developed for reversed-phase separations and this may restrict the extent to which the technique is adopted.

Recent studies in thin films provide a glimpse of the role thin-layer chromatography might play in the world of miniaturized separation techniques. The separation media is still in the development phase for these methods and their performance is difficult to judge because the instrumentation required for their efficient use is not adequate at present. Until advances in instrumentation and layer development become equalized the prospects for ultra thin-layer chromatography remain undecipherable.

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