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Characterizing Dispersion in Microfluidic Channels

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Abstract

Dispersion or spreading of analyte bands is a barrier to achieving high resolution in microfluidic separations. The role of dispersion in separations is reviewed with emphasis on metrics, sources and common principles of analysis. Three sources of dispersion (a) inhomogeneous flow fields, (b) solute wall interactions and (c) force fields normal to channel walls are studied in detail. Microfluidic and nanofluidic applications to capillary electrophoresis, chromatography and field-flow fractionation, that are subject to one or more of these three physical processes under standard, unintentional or novel operating conditions, are discussed.

I. INTRODUCTION

In a “Lab-on-a-chip” device, the capability for performing a number of basic fluidic processes needs to be integrated onto a single platform. Perhaps the most important of these are (a) mixing (b) separation. In the case of (a), the problem is, how does one homogenize two species carried by a flow efficiently in the absence of turbulence, which greatly enhances mixing processes in large scale systems? In the case of (b), the problem is quite the opposite; how does one prevent the different species in a flow from diffusing into each other and reducing the efficiency of the separation mechanism? Paradoxically, in microfluidics both problems are hard! This brief review is devoted to the exposition of the underlying physical principles that govern separation efficiency. Molecular diffusion, which is a macroscopic manifestation of the underlying microscopic process of random thermal motion, clearly aids the process of homogenization. It is therefore an ally in our efforts to design a good microfluidic mixer. One would naturally assume that it is the principal enemy in the case of separation processes. But dispersion mechanisms can be subtle, and we will see in the next section, that this is often but not always the case! It is because of these subtleties of the underlying physical processes that the practical engineer needs to be aware of some aspects of the science of dispersion, which is the focus of this article.

Dispersion of a solute in a liquid or gas refers to processes, that, in time, lead to the spreading of the solute distribution; that is, increasing the variance in spatial positions taken by a group of particles around their mean position. Molecular diffusion is an obvious microscopic mechanism of dispersion that is omnipresent and unavoidable. However, macroscopic processes, such as mass exchange between phases or nonuniform fluid flow can greatly affect [1] the effectiveness of molecular diffusion in causing dispersion. It is through controlling these aspects that the designer tries to control the efficiency of separation. An understanding of the factors that lead to dispersion have contributed to progress in various branches of science and

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technology; for example, the accurate measurement of diffusion coefficients [2,3], the transport of colloidal particles and soluble salts in porous media and rock fractures [4,5], silt transport by rivers [6] and more recently, the design of efficient separation processes [7,8].

Dispersion may arise due to spatial variations in fluid velocity or due to interphase mass transfer (chromatographic dispersion [*cf.* 1]). The factors which cause dispersion often simultaneously cause skew or asymmetry in the solute distribution, at least at short times from the introduction of the solute in a fluid. For example, Figure 1 shows a visualization of an experiment [9] where an inert dye is transported in a microchannel by a liquid driven by a pump. Since this pressure driven flow has a parabolic profile, an initially uniform plug of dye is pulled into a “bullet shape” by the flow. Thus, strong density gradients in the radial direction are created, which, even in the presence of a small molecular diffusion tend to homogenize the dye over each cross-section. The overall effect is an enhancement of the rate of axial dispersion over and above what would occur due to molecular diffusion alone in the absence of flow. The phenomena is called “shear-induced” or “Taylor” or “Taylor-Aris” dispersion, [*cf.* 2,10]. Perhaps counterintuitively, the smaller the molecular diffusion coefficient, the larger is the dispersion produced by flow shear. Since Taylor dispersion and ‘ordinary’ molecular diffusion in the axial direction act together, minimum axial dispersion is realized at an intermediate value of the diffusion coefficient.

A primary goal of chemical and biochemical separations is to reduce the time of analysis; this is fundamentally enabling for a variety of applications such as point-of-care medical diagnostics [11], fast sequencing of genomes [12], detection of environmental contaminants [13]. However, it is equally important to obtain a strong, conspicuous, well-defined signal for a given analyte unmasked by extraneous factors such as background noise, contaminants, or other analytes [14,15] from the sample undergoing separation. Ensuring the last condition involves control of analyte dispersion.

We consider a separation taking place in a capillary or a channel with a detection port separated from the sample injection port by a distance L (Figure 2). For a microchannel separation, L is of the order of several centimeters and the channel width is of the order of several tens of microns. Examples of separations taking place in this format are capillary zone electrophoresis, capillary electrochromatography, liquid chromatography. Below, we define certain metrics to quantify separation efficiency using a simple approach suggested by [16]. The particular mechanism of the separation is unimportant in the discussion below, but we utilize terminology from the theory of chromatographic separations. For example, the term ‘column’ is used to indicate the part of the separation channel between the injection and detection ports. The plot of analyte concentration with time at the detection port is called a chromatogram (*cf.* Figure 3) [1].

During the time it takes to migrate the distance L on Figure 2, the sample separates into bands consisting of its different constituents. Each constituent has a unique characteristic retention (or detection) time, t_r which is the time between the injection of sample (start of separation) and detection of the particular constituent’s peak in the chromatogram. Additionally an inert analyte which moves with the mean speed $\bar{u}$ of the fluid flow in the separation device has a detection time of t_0 . The ratio:

$$k = \frac{t_r}{t_0} - 1 \quad (1.1)$$

is called the capacity factor [18]. The standard deviation $\langle(\Delta t_r)^2\rangle^{1/2}$ (in units of second) of an analyte peak in the chromatogram at the time t_r of its detection can be used to quantify

dispersion in a separation. The plate number is a dimensionless measure of column efficiency defined as:

$$N = t_r^2 / \langle (\Delta t_r)^2 \rangle \quad (1.2)$$

The shape of the analyte's axial distribution when its peak passes the detector is usually a good surrogate for its chromatogram near its retention time t_r . Using the standard deviation of the spatial distribution, $\sigma_x = \sqrt{\int x^2 c dV / \int c dV}$ (unit is that of length, x is the direction of separation, c is the local concentration of the analyte and the volume integral is over the volume of the solute in the capillary) at the instant of detection, the Plate Number (N) can also be calculated from:

$$N = L^2 / \sigma_x^2 \quad (1.3)$$

N can be of the order of 10^4 for conventional high performance liquid chromatography columns [19]. In many separation applications [20], the spreading of a moving band of analyte can be regarded as a diffusive process about its current mean position; one characteristic of such a process is that the standard deviation σ_x is proportional to the square root of time [10,21]. If the analyte migrates with a fixed speed v_m for time t_r before it is detected at a distance $L = v_m t_r$ from its point of injection, then, $\sigma_x^2 \propto t_r \propto L$, so that $N \propto L$ in Equation (1.3), which implies that a long separation column is characterized by a larger value of the plate number N than a shorter column. Therefore,

$$H = \frac{L}{N}, \quad (1.4)$$

the Height Equivalent of Theoretical Plate (HETP) or plate height (H) is a more meaningful measure when comparing separation columns of different lengths. For efficient separations, H should be small and is typically a few millimeters or less for capillary realizations of liquid chromatography, cf. Figure 3 of [22] or Figure 13 of [23].

The effective dispersion coefficient D_{eff} defined as:

$$D_{eff} = \frac{1}{2} \frac{d\sigma_x^2}{dt} \quad (1.5)$$

as a way of characterizing the spread of analyte is often more amenable to theoretical treatment. If initial transients are short, the variance of an analyte detected at time t_r approaches the asymptotic form [10]:

$$\sigma_x^2 \simeq 2D_{eff}t_r \quad (1.6)$$

Rewriting Equation (1.4) with the help of Equations (1.1), (1.3), (1.6) and the definition $L = \bar{u}t_o$:

$$H = \frac{2D_{eff}(k+1)}{\bar{u}} \quad (1.7)$$

The performance of the column in a particular separation can be measured by its resolution factor R_s . If the labels 1 and 2 are used for two consecutively eluting analytes, R_s can be defined as [24]:

$$R_s = \frac{(t_{r1} - t_{r2})}{2(\sigma_1 + \sigma_2)} \quad (1.8)$$

The standard deviations $\sigma = \langle (\Delta t_r)^2 \rangle^{1/2}$ in Equation (1.8) are measured in time, as in Equation (1.2).

For optimizing separations, the separation media, mechanism and geometry need to be chosen to have a low t_r , high R_s and low H . Low separation time ($\propto t_r$) can be achieved by increasing the flow speed $\bar{u}$ or the migration speed of analyte. Dispersion mechanisms, the main focus of this review, should be characterized and controlled to achieve low H . However, the metrics t_r , R_s and H are inter-related and depending on the separation objectives, methods, geometry and independently controlled variables, a wide variety of trade-offs may be involved in designing an efficient separation [16,25–27].

For example, an attempt to reduce the separation time by increasing the flow speed $\bar{u}$ or the migration speed of the analyte may lead to a significant rise in H for pressure-driven separations and separations based on the chromatography principle (as discussed later in this review). In separations utilizing electroosmotic flow, such as capillary electrophoresis with a homogeneous buffer solution and homogeneous channel walls, the applied voltage can be increased to increase the migration speed of the analyte without significantly affecting H only up to the point where the undesirable effects of Joule heating sets in [28].

For analyte migrating at a given speed, R_s can be increased by increasing the distance L , but at the cost of a longer separation time as well as larger voltage/pressure drops [26]. Given a specific pair of consecutively eluting analytes with sufficiently different capacity factors k , R_s can instead be reduced by a reduction in H (since $\sigma \propto \sqrt{H}$) for both the analytes. Since the signal from an analyte peak at the detector deteriorates as the peak widens, a separation with low H (and low t_o) also has the advantage of being able to detect low analyte concentrations.

From the above discussion, it is evident that reduction of band broadening as measured by H is pivotal for efficient separations. The factors affecting band broadening or dispersion will be the subject of the remainder of this review. The choice of separation methods and separation media have a significant bearing on the resolution and efficiency of separations [29,30] but are outside the scope of this review.

The organization of this review is as follows. Section II is devoted to reviewing the principles behind differential migration of analytes in various separations. In Section III the three sources of dispersion present in chromatographic, electrophoretic and field-flow-fractionation methods are discussed. These are, inhomogeneities in the flow field, interaction of the analyte with the channel walls and/or effect of external force fields normal to the wall. There are various other important sources of dispersion in microfluidic systems, like dispersion induced by turns in a microchannel [31,32], Joule heating [28,33,34] and high sample concentration affecting buffer conductivity [35]. These dispersion modes have been reviewed in detail elsewhere [36,37]. Conclusions are presented in Section IV.

II. ANALYTE MIGRATION IN MICROFLUIDIC SEPARATIONS

In order to separate from a sample, molecules of different analytes should at least have different speeds in the initial stages of a separation. Further, in order to ensure that the bands are not overwhelmed by dispersion in the long run, the analyte bands must continue to move with speeds that are different from one another. The retention ratio R can be defined as the ratio of the eventual migration speed of the analyte to that of an inert solute (not interacting with the separation system) and can be used to characterize analyte migration behavior. By this definition, R can also be related to the capacity factor as $R = 1/(1 + k)$. Each analyte should have a distinct retention ratio based on a distinct property of its interaction with the separation system. Exceptions are separation such as isotachopheresis and capillary isoelectric focussing [38] where self-sharpening rather than dispersive spreading is in action; different R values are not necessary as long as adjacent bands can be kept apart from each other.

Table I summarizes the retention ratios for several microfluidic and nanofluidic separations. The acronyms GC, LC, GPC and EKSIV stand for gas chromatography, liquid chromatography, gel permeation chromatography and Electrokinetic Separation by Ion Valence respectively. The ratio R is the retention ratio for the analyte in the separation. The axial coordinate of the separation channel is x , the coordinates in the cross-sectional plane are y, z . A bar over any quantity denotes its averaging across the cross-section of the channel. The shape of the fully developed axial velocity $u(y, z)$ profile in the channel is given by $u = \bar{u}F(y, z)$, so that $\bar{F} = 1$ by definition. The electrophoretic and electroosmotic mobility are μ_{ep} and μ_{eo} , respectively. $E(y, z)$ is the potential energy due to the normal field force used in field-flow fractionation. In GC, LC, GPC and capillary electrochromatography, k is the ratio at thermodynamic equilibrium of the number of molecules in the stationary phase to that in the mobile phase. In hydrodynamic chromatography, K_c is a function of the ratio λ of particle to channel width characterizing the hindered convection of large molecules inside the channel, known as ‘hindered convection coefficient’. For a spherical molecule of radius a in a cylindrical channel of radius R_0 and in a slit-shaped nanochannel of width $2h$, $\lambda = a/R$ and $\lambda = a/h$ respectively. In EKSIV, Z is the valence of analyte, ϕ is the electric potential in the electrical double layer due to a charged nanochannel wall e is the elementary charge, k is the Boltzmann’s constant and T is the temperature.

Below, we briefly discuss the migration mechanisms in the separations referred to in Table I, see also the references mentioned on this table. In conventional liquid or gas chromatography (first row of Table I), separation is effected by allowing the molecules to make intermittent trips from the mobile phase to the stationary phase which slows different species to different degrees. In hydrodynamic chromatography, a pressure-driven flow which has a parabolic velocity profile with the highest velocity at the channel center and the lowest (zero) on the wall is used. Large colloidal particles carried by the flow are sterically excluded from the region near the walls which is also a region of slow fluid motion. Therefore, larger solutes actually move faster than smaller solutes which can sample all the streamlines in a flow. In capillary electrophoresis, solutes with different charges acquire different migration velocities under an electric field through the combined action of electrophoresis and electroosmosis. Capillary electrochromatography uses an electric field to move the analytes while retaining an adsorbent stationary phase similar to chromatographic techniques. In field-flow fractionation (FFF) techniques, a normal force (such as flow normal to porous wall, electric field) is used to distribute the sample molecules closer to the channel walls in a pressure-driven flow which results a reduction in elution speed compared to a situation where the normal force is absent.

In nanochannels of width comparable to the Debye layer thickness of the background electrolyte solution, the electrostatic attraction/repulsion between the charged walls and the charged sample constituents can naturally exert a fractionation effect in the wall normal

direction. Independent determination of the valence and the electrophoretic mobility of ions based on this principle was demonstrated by [39,40], while axially transporting the analytes using an applied electric field as in capillary electrophoresis; this separation was termed Electrokinetic Separation by Ion Valence (EKSIV). The retention factor in EKSIV can be calculated by adding the retention factors for capillary electrophoresis (second row of Table I) to that for field flow fractionation (last row) with the choice of $E = Z\phi E$ representing the electrical potential energy of a small Z -valent ion within the electrical double layer and the function F chosen to represent the electroosmotic flow profile in the nanochannel. The fractionating effect of Debye layers in nanochannels has been elsewhere termed “autogenous electric field-flow fractionation” [41].

III. SOURCES OF DISPERSION IN MICROFLUIDIC SEPARATIONS

A. Inhomogeneities in the hydrodynamic flow field: application to electrokinetic and pressure-driven separations

1. Dispersion in pressure-driven flows: short and long time limits—The most well-studied example of dispersion to date is that for a solute transported by a pressure-driven flow; *cf.* Figure 1, the primary references [2,10,50] and the reviews [5,6,51]. There is a significant difference in the spreading behavior of the solute shortly after its injection and after several diffusion times have elapsed after its injection, as discussed below. The latter has been a traditional focus area outside the realm of microfluidics, however transport in microfluidics can often be fast enough for the “short time regime” to be appropriate [7]. Below, we assume pressure is being used to drive the flow as in Figure 1 and present a short discussion on these two regimes.

The characteristic axial width of the sample zone increases with time. In a channel of width $2h$ carrying a species with diffusion coefficient D in the background electrolyte, the characteristic time of cross-channel diffusion is $t_d = h^2/D$. If during this time the sample has migrated a distance L with an average speed U_0 , an axial convection time scale can be defined as $t_c = L/U_0$. The regimes $t_c \ll t_d$ (known as the ‘short time regime’ or ‘ballistic regime’) and $t_c \gg t_d$ differ with respect to the rate at which the solute spreads. In the short time regime visualized in Figure 1, the cross-channel diffusion is yet to take effect and the axial gap between the fastest moving sample at the channel center the stagnant particles on the wall stretches linearly with time. As a consequence, it turns out that the axial spread of the distribution (characterized for example by the standard deviation σ) also increases linearly with time ($\sigma \propto t$) [52–55]. After a sufficiently long time has elapsed for cross-channel diffusion to take effect ($t_c \gg t_d$), the sharp edges of the paraboloid in Figure 1 fray to produce a blob of die which is nearly uniform across the channel and the plug spreads (about its moving centroid) with a characteristic width proportional to the square root of time ($\sigma \propto t^{1/2}$), a scaling which also characterizes pure diffusion. In this regime, first identified and studied by Taylor [2], the spreading of the solute can be analyzed by a constant ‘effective dispersion coefficient’.

2. Methods for predicting dispersion—In his seminal work on spreading of solutes in pipes, Taylor [2] calculated that the spreading rate of an inert solute about the mean speed is inversely proportional to its molecular diffusion coefficient and presented an approximate analysis which was valid for slowly diffusing solutes and in the long time limit. Aris [10] removed the restriction of slow diffusion and also put Taylor’s work under a more general and rigorous theoretical framework known as the Method of Moments.

In analyzing dispersion with the Method of Moments, the main quantity of interest is the axial variance of the analyte distribution, which is the second statistical moment of the analyte distribution about the axial coordinate around its centroid. The ‘zeroth moment’ is the current fraction of analyte, and the ‘first moment’ is the axial position of the centroid of the analyte

distribution. In the form studied by [10], a local moment is defined at each transverse elemental volume of a straight channel (*e.g.* for a cylinder, this elemental volume is a ring-shaped filament extending all along the channel at each radial location). An integral moment for the entire volume of the channel is finally defined as the integral of the local moments over all radial stations. By suitably integrating the equations of mass transfer, the recurrence relation at the heart of this method which relates the integral moment of a given order to the local and integral moments of the previous order is obtained. The variance, or the integral second moment about the centroid is the chief quantity of interest in a dispersion calculation. To obtain the variance, the zeroth and first order local moments have to be known first. Performing the steps outlined above, Aris obtained an expression of the following form for the effective dispersion coefficient valid in a straight channel after several cross-diffusion times (*i.e.* in the long time limit):

$$D_{eff} = \frac{1}{2} \lim_{t \rightarrow \infty} \frac{d\sigma^2}{dt} = D + \gamma \frac{h^2 \bar{u}^2}{D}. \quad (3.1)$$

In the above equation, h is a characteristic width of the channel, D is the molecular diffusivity and $\bar{u}$ is the cross-sectionally averaged axial flow velocity. The numerical prefactor γ in Equation (3.1) will be called dispersivity in this article. The dispersivity γ depends on the flow profile in the channel and the shape of the channel cross-section. For example, for circular

capillaries and pressure-driven flow $\gamma = \frac{1}{48}$, if h is taken as the capillary radius. The calculation of dispersion in microfluidics can often be reduced to obtaining a value/expression for γ under the geometry and flow field of interest (*cf.* [55, 56]). The term ‘Taylor dispersivity’ and the notation γ_p will be used in this article, where situations will arise to distinguish between the dispersivity under other flow types (*e.g.* electrokinetic) and pressure-driven flow under the same channel geometry.

The Method of Moments has been used to study dispersion in subjects as diverse as porous media and transport of silts in rivers and estuaries in addition to dispersion in microfluidic systems, see [57] for a broad overview. It should be noted that in the ‘Method of Moments’, one does not attempt to resolve either the axial or the transverse analyte distribution within the longitudinal filaments that one investigates.

Other methods, by which effective dispersion coefficients can be ultimately calculated in the same general form as Equation (3.1) include multiple scale asymptotics (Fife and Nicholes [50]), a ‘Reynolds decomposition’ [58] type mean and fluctuation method [5] and the dynamic theory of center manifolds [59, 60]. All these methods take advantage of the fact that cross-diffusion of the solute in the narrow channel is much faster than the axial spreading. Another feature that these approximate methods share with the Taylor’s original analysis (and not with the Method of Moments in its traditional form [10]) is that the cross-channel concentration distribution in the long time limit can be predicted as a by-product of the analyses. These methods are particularly suitable for microfluidics because the small width ($\sim \mu m$) to length ($\sim mm$ or larger) ratio of microchannels.

3. Dispersion under slowly varying electrokinetic flows—In capillary electrophoresis (CE) in microchannels a plug-like electroosmotic flow profile transports charged and inert solutes making it an inherently low dispersion technique. However, incidental/intentional variations in conditions such as the wall zeta potential, cross-sectional area or the conductivity of the solution can induce pressure-gradients in the electroosmotic flow making the electroosmotic flow profiles both axially and radially inhomogeneous and contributing to dispersion [31,36,61]. Dispersion in CE under axially variable electroosmotic flow can be studied either through complete three-dimensional numerical simulation, or through an

asymptotically reduced description valid in the long time limit that takes advantage of the small width to length ratio of a microchannel and requires only one-dimensional simulations [61, 62]. A detailed discussion on this asymptotically reduced description will appear in a later section, where effects of analyte-wall interactions will also be discussed.

As an example, Figure 4 from [62] shows effective axial diffusivities for electroosmotic transport of a passive scalar by an axially variable electroosmotic flow pattern generated by a sinusoidally varying wall zeta potential in a square channel of width $2b$. The controlling variables here are the amplitude ($\Delta\zeta$) and wavelength Λ of the periodic zeta potential $\zeta(x) = \langle\zeta\rangle + \Delta\zeta \cos(2\pi x/\Lambda)$, where x is the coordinate along the axis of the channel and $\langle\zeta\rangle$ is the axial average of the zeta potential. In the figure, comparisons are made between a fully resolved 3D numerical calculation and a one-dimensional reduced model based on the lubrication

approximation. The effective dispersion coefficient $D_{eff} = \frac{1}{2} \frac{d\sigma^2}{dt}$ is extracted from a full three-dimensional solution of the electroosmotic flow. The investigations [62,63] showed that in practice the reduced one-dimensional models are highly accurate even when $\Lambda \sim 2b$, although the formal validity of the corresponding asymptotic analyses require $\Lambda \gg 2b$.

4. Dispersion under axially uniform flow patterns—The values of dispersivity for several axially uniform flow patterns in a plane channel are shown in Table II. Pressure-driven flow is used in liquid-chromatography and electroosmotic flow is used in capillary electrophoresis. Couette flow or flow of a viscous fluid in the gap between two plates is a good approximation to the flow pattern in shear-driven chromatography [64] and separation by AC electroosmosis [65]. For a solution containing a binary salt (dissociating into monovalent ions) of concentration n_0 moles per unit volume the Debye Hückel parameter (κ) appearing in Table II and the Debye length λ are defined as:

$$\kappa = \frac{1}{\lambda} = \sqrt{\frac{\epsilon k T}{2 n_0 e^2 N}} \quad (3.2)$$

where ϵ , k , T , e and N are the electrical permittivity of the solution, Boltzmann's constant, temperature, electronic charge and Avogadro's number respectively.

5. Effect of channel cross-sectional shapes—The third row of Table III shows calculated value of γ for various cross-sectional shapes. It is essential to know the precise cross-sectional shape to obtain the value for γ . For example, we can note from Table III, that the Taylor dispersivity for a channel with square cross-section is about $0.0084 \times 192 \approx 1.61$ times that of a capillary with cross-section in the shape of its inscribed circle; the larger value for square is consistent with the presence of higher shear in the corner regions of the square. Note that, the value of the Taylor dispersivity is sometimes quoted in the literature [10, 42, 67] as $1/48$ for capillaries of circular cross-sections and $2/105$ for plane channels. Our values in Table III differ from those values, because our values are referred to the diameter of the capillary (in place of the radius) and the width of the plane channel (in place of the channel half-width).

The review by [56] is focussed extensively on the effects of cross-sectional shape as applied to microfluidics. We emphasize below some interesting aspects of the effect of cross-sections as discussed in the literature. We emphasize below some interesting aspects of the effect of cross-sections as discussed in the literature.

Channels with small depth (d) and large width (w), such that $d \ll w$, often result from microfabrication technologies. In characterizing dispersion for time $t \gg w^2/D$ using Equation

(3.1) (with $h = d$) for such a channel, care should be exercised to employ the Taylor dispersivity for rectangular cross-section in the small aspect ratio limit [56] and not the value $\gamma = 1/210$ appearing on Table III. The former is about 7.95 times that of the latter because the region over which the axial velocity drops to zero on the side walls of the rectangle introduce a finite dispersion in the flow [6, 56, 68]. On the other hand, $\gamma = 1/210$ will describe the dispersion in the same channel during the intermediate time regime $d^2/D < t < w^2/D$, since in this regime the widthwise velocity gradients are yet to be sampled by the analyte [4]. For wide microfluidic channels with gradual rather than abrupt change in depth (as in a rectangle), fabricated e.g. by soft lithography on PDMS [69], scaling arguments to estimate dispersion in the long time limit should be based on the width w rather than the depth d of the channel [55].

In some experimental situations, it may be necessary to judge the relative significance of Taylor dispersion and molecular diffusion effects. The ratio of the second term of Equation (3.1) to its first term is γPe^2 , where $Pe = \bar{U}h/D$. In a microchannel of depth $h = 10 \mu\text{m}$ and width $w = 500 \mu\text{m}$, carrying a large biomolecular solute in aqueous solution with $D \sim 10^{-10} \text{ m}^2/\text{s}$ and a cross-sectionally nonuniform flow of 0.1 mm/s , $Pe \sim 10$; therefore, the contribution from Taylor dispersion to the total dispersion is about 50%.

B. Interactions with channel wall: chromatographic and proteomic applications

The analyte can have desirable and/or undesirable interactions with the wall of the channel or, perhaps other solid substrates such as packing, beads, fibers, gels that are part of the separation system [1]. One of the effects of such wall interactions is to increase the degree of dispersion. Solute interactions may be classified as “irreversible” or “reversible”. In the latter case, most of the analyte manages to return to the solution through desorption during the separation process. Irreversible processes, where the desorption can be neglected can be fast or slow.

A widespread problem in separation of proteins and peptides is the tendency of these molecules to adsorb to any charged surface, the so called ‘nonspecific adsorption’ problem [70,71]. Protein adsorption is typically a slow and irreversible process. Protein adsorption in electrokinetic separation can modify the surface electrokinetic properties and create an axially and radially varying electrokinetic flow field leading to increased dispersion by the mechanism discussed in the section on slowly varying electrokinetic flows [61,72]. A fast irreversible adsorption involves even larger loss of analyte and is of limited interest, unless the surface adsorbed species itself is being detected, as in immunoassays and biosensors [73].

Problems with adsorption and desorption can be addressed in a general manner, by a combined model of the liquid-phase electrokinetic/pressure-driven transport with the chemical kinetics of the system. A full three-dimensional numerical solution of flow and analyte concentration fields are of course possible, but is a laborious option on account of the high length to width aspect ratio of microfluidic channels. It is much more efficient to take advantage of the high aspect ratio to bring about an asymptotic reduction to a one-dimensional problem involving only the axial co-ordinate and time as independent variables. Such an approach has been developed for a wide class of dispersion problems in microfluidics including those involving reversible and slow irreversible adsorption kinetics. Since majority of microfluidic separations are conducted with an open channel architecture [74] (*i.e.* without any packing, gels *etc.* inside the channel, *cf.* Figure 2), the discussion below is limited to situations where the adsorptive surface/coating, if any, lies on the walls of the microchannel.

An effective set of reduced order equations for calculating the area average $\bar{c}$ of the liquid-phase analyte concentration c as a function of time t and axial coordinate x for a channel of constant cross-section is (*cf.* [61,62]):

$$\bar{c}_t + \bar{u}\bar{c}_x = -\alpha\bar{s}_t + [(D + \gamma\bar{u}^2 h^2 / D)\bar{c}_x]_x - \frac{h^2 \bar{u}}{D} (\alpha\beta\bar{s}_t)_x \quad (3.3a)$$

$$\frac{\partial s}{\partial t} = f(c_w, s) \quad (3.3b)$$

$$c = \bar{c} + \frac{\bar{u}c_x}{D}\chi - \alpha\frac{s_t}{D}\psi \quad (3.3c)$$

where α is the ratio of perimeter of the channel cross-section to its area, h is a characteristic width of the channel and D is the diffusion coefficient. The axial velocity component u of the flow field in the channel is allowed to vary axially on a length scale much larger than the channel width [75], as well as radially. ψ and χ are functions defined below. A subscripted x and subscripted t indicates differentiation with respect to these variables. The variable s is the surface concentration of solute or the number of moles of solute adsorbed per unit area of the surface (here assumed not to have any perimetric variations).

A bar placed over any quantity defined on the cross-section of the channel indicates its average over the cross-section of the channel. The subscript w on any quantity indicates that the quantity is evaluated on the curve bounding the two-dimensional channel cross-section (*i.e.* on the wall). A bar placed over a quantity with subscript w will indicate its average over the boundary curve on which it is defined *i.e.* its perimetric average. The parameters $\beta = \bar{\chi}_w / h^2$ and $\gamma = \overline{\chi u} / h^2$.

The function $f(c_w, s)$ describes some single-step chemical kinetic law describing the adsorption-desorption on the wall. When the adsorption sites on the wall are too numerous to saturate, the adsorption rate is proportional to the concentration c_w evaluated on the wall from the liquid side (linear kinetics). If the number of adsorption sites is finite, so that the surface can saturate, the adsorption rate follows the nonlinear ‘Langmuir law’ (*cf.* [72]) being proportional to the product of c_w and the current number of unoccupied sites. Adsorption is usually accompanied by a desorption process, unless the reaction is essentially irreversible.

Since transport is volumetric and adsorption occurs over a surface, the surface to volume ratio of the channel α enters the problem. Additionally, a function $\psi(y, z)$ characterizes the effects of surface adsorption on the cross-channel variation of concentration (Equation (3.3c)) and can be calculated by solving:

$$\psi_{yy} + \psi_{zz} = 1 \quad (3.4a)$$

$$[\psi_y m + \psi_z n]_w = \frac{1}{\alpha} \quad (3.4b)$$

where $\bar{\psi} = 0$, (m, n) are the direction cosines of the curve bounding the two-dimensional channel cross-section described by the variables y and z at a given axial location ‘ x ’. The subscripts with y and z denote differentiation, the order of differentiation being indicated by repetition. The function χ is dimensionless and is defined by

$$\chi_{yy} + \chi_{zz} = \frac{u}{u} - 1 \quad (3.5a)$$

$$[\chi_y m + \chi_z n]_w = 0 \quad (3.5b)$$

Here, once again $\bar{\chi} = 0$ and u is the axial flow velocity. Table III tabulates β , γ and δ for various geometries. The parameters $\beta = \gamma = 0$ for channels with uniform flow.

The special case of Equation (3.3) with $s = 0$ can be used to study the approximate effect of flow fields that are nonuniform axially and radially, provided axial variations are on a scale much larger than the channel width. The asymptotic model referred to in Figure 4 and the corresponding discussion in Section IIIA3 is precisely Equation (3.3) in this limit, with the electroosmotic flow field under the axially variable zeta potential calculated using the lubrication approximation [75].

Considerable simplifications to Equation (3.3) are possible in presence of geometric symmetries [72], knowledge of certain characteristics of the wall adsorption-desorption kinetics [62] and the flow type [76]. To further understand wall interactions, the role of different types (irreversible and reversible) of adsorption-desorption are studied briefly below; further details appear in [62]. As mentioned previously, the problem of irreversible adsorption is relevant to biosensing and proteomics; reversible adsorption-desorption is fundamental to chromatographic separations.

1. Irreversible adsorption—Irreversibly adsorbing analytes move faster and disperse slower than inert analytes in pressure-driven flow [77,78]. This is because, the slow-moving analyte molecules near the wall, which are also subject to the highest flow-shear-induced deformation and spreading are preferentially removed from the channel. For electrokinetic flows, the analyte migration and dispersion is significantly affected only if the adsorbed solute alters the zeta potential of the wall [61]. Equation (3.3) was applied to the protein adsorption problem in capillary electrophoresis by [72] to obtain realistic peak spreads and shapes.

Irreversible adsorption processes that are so fast as to be essentially complete before analyte can migrate to the detector are less relevant to analytical techniques such as CE and LC which detect solutes from the liquid phase. Because of the large sample loss incurred in irreversible adsorption (which may even take place within an axial distance which is only a few channel widths downstream of the injection port [70]), the solute may no longer be detectable at the analysis port. However, certain analytical techniques such as affinity chromatography and solid-phase-extraction depend on detecting the analyte after adsorption in the solid phase and these have applications in immunoassays, biochemical hazard detection and medical diagnostics [79]. These techniques may employ a fast irreversible adsorption for the purpose of reducing analysis times. To characterize analyte transport under such conditions (other than through direct numerical solution), interest lies (at least) in the period where the cross-diffusion is still competing with the transient effects arising from the analyte loss from the liquid phase. Irreversible adsorption is also appropriate for describing situations where heat, not mass, is lost through the solute stream. See for example the analysis by [78] where both fast (as well as slow) irreversible adsorption (in the form of heat loss) is included in calculating both the effective migration speed and the effective dispersion coefficients.

2. Reversible adsorption-desorption—If desorption of analytes from the wall back into the solution is a significant process in the early stages of a separation, the analyte in the liquid

phase will come to an approximate chemical equilibrium with the adsorbed product species; however, a small degree of non-equilibrium must still prevail to sustain the transport processes in the channel. A significant consequence of the approximate equilibrium mentioned above is that analytes which manage more ‘trips’ to the solid phase will move slower; this is the principle of chromatography. However, the small departure from the equilibrium that remains is the primary cause of the chromatographic band broadening harmful to separation efficiency. The earliest rigorous studies of chromatographic band broadening was done by [42,80].

Numerical solution of Equation (3.3) can be used for calculating the effective dispersion coefficient in a time dependent manner. However, for linear adsorption-desorption kinetics, based on the observation of small departures from equilibrium, an analytical expression for the effective dispersion coefficient valid after sufficient time has elapsed for desorption to nearly equilibrate with adsorption can be obtained for either through direct simplification of the full equations of solute transport [80], or through simplification of Equation (3.3) [62]. The corresponding plate height, as defined in Equation (1.7), for a uniform channel of arbitrary cross-section carrying an arbitrary axially developed flow field is

$$H = \frac{2(1+k)}{\bar{u}} D_{eff}^{(chrom)} = 2 \left[\frac{D}{\bar{u}} + C \frac{h^2 \bar{u}}{D} + \frac{k}{k_d(1+k)^2} \bar{u} \right] \quad (3.6a)$$

$$C = \gamma + \frac{2k\beta}{1+k} + \frac{k^2\delta}{(1+k)^2} \quad (3.6b)$$

where k_d is the desorption rate constant and k is the capacity factor, $\gamma = \overline{\chi u}/h^2$, $\eta = \overline{\chi_w}/h^2$ and $\delta = \overline{\psi_w}/h^2$ as introduced earlier. The quantity C is known as the ‘mobile phase band broadening factor’ or the ‘C term’ in chromatography practice [1]. The capacity factor k in a chromatographic process is related to the equilibrium constant K (ratio of adsorption rate constant k_a and k_d) by $k = \alpha K$. The ‘C term’ carries contributions from Taylor Aris dispersion (first term of Equation (3.6b)), interaction between adsorption-desorption and flow (second term of Equation (3.6b)) and adsorption-desorption alone (third term of Equation (3.6b)). The last term of Equation (3.6a) arises from the slowness of the adsorption-desorption process and can be neglected near chromatographic equilibrium if the kinetics is fast compared to the transport time. The Method of Moments was used by Aris [80] to obtain a variant of Equation (3.6).

Increasing the speed of flow is an obvious strategy to reduce the time of analysis in chromatography. However, it is clear that too large flow rates can make the second term of Equation (3.6a) large leading to undesirably large plate heights. Thus, optimization of chromatographic systems require understanding of the ‘C term’ (Equation (3.6b)).

Equation(3.3) can be specialized to different flow patterns (such as pressure-driven, shear-driven and electroosmotic) through specification of the two-dimensional flow shape function $u/\bar{u}$ (cf. Table II) and to different cross-sectional shapes through specification of the shape factors δ , β and γ (cf. Table III). The ‘C term’ has been tabulated for open-channel modes of various chromatographic separations such as pressure-driven liquid/gas chromatography, electroosmotically-driven capillary electrochromatography (CEC) [81], shear-driven chromatography (SDC) [64] in Table IV. Capillary electrochromatography (CEC) is performed with a plug-like electroosmotic flow profile in microchannels with adsorbing walls. It can be noted from Table IV (or by setting $\gamma = \beta = 0$ in Equation (3.6b)) that CEC is not dispersion-free [82]. In addition to the contribution of the ‘C term’, the contribution of kinetics to zone

broadening (the last term of Equation (3.6a)) is also present in CEC, if the time scale of adsorption-desorption is of the same order as the transport time. Setting $\delta = \beta = 0$ (or $k = 0$) for non-adsorbing walls, Equation (3.6b) gives $C = \gamma$ corresponding to an inert solute undergoing Taylor Aris dispersion.

For predicting the pre-equilibrium stages of dispersion before Equation (3.6) becomes accurate, either Equation (3.3) or the full transport problem needs to be solved. In Figure 5, the transport of a neutral analyte injected as a Gaussian plug in an EOF with thin Debye layers established within a channel of square cross-sectional shape is studied. The effective dispersion coefficient D_{eff} is calculated at a given detection time with the approaches based on these three equations for reversible wall interaction and advective-diffusive transport in a channel of square cross-section under electroosmotic flow resulting from a uniform wall zeta potential. The quantity λKPe on the horizontal axis can be interpreted as the ratio of characteristic times for adsorption and diffusion. The dimensionless adsorption rate constant λK and the dimensionless desorption rate constant λ are varied keeping the dimensionless equilibrium constant K fixed, which means that the speed of the reversible wall reaction increases from left to right on Figure 5. For all reaction speeds, the 1-D model based on Equation (3.3) furnishes almost the same information as the 3-D model, but at a much lower computational cost. For slow reactions (small values of λKPe), the chromatographic model based on Equation (3.6) is inaccurate as the quasi-equilibrium conditions required for the validity of chromatographic dispersion theory [83] cannot be approached under the finite detection times investigated. For sufficiently fast reactions, the dispersion coefficient becomes independent of the speed of reaction, as evident from either Figure 5 or the smallness of the last term of Equation (3.6a) under this condition. Figure 5 serves to validate and highlight the advantages of the approach based on the 1-D effective equation (Equation (3.3)) vis-à-vis either the solution of full transport equations or the use of the analytical expression for chromatographic dispersion coefficient (Equation (3.6)) in investigating microfluidic systems ($h \ll L$) with adsorption-desorption on walls, when the elapsed time t satisfies $t \gg h^2/D$, but not necessarily $t \gg h/k_a$, and $t \gg 1/k_d$. A video of the time evolution of the average concentration distribution, $\bar{c}(x, t)$ of an analyte moving in a rectangular channel under an uniform electroosmotic flow and interacting with the wall according to a slow but reversible adsorption-desorption kinetics is included in the supplementary material. The initial stages of this simulation is characterized by an asymmetric peak shape that results from the trailing edge of the analyte plug receiving more desorbed solute molecules than the leading edge, and therefore, moving more slowly; this peak feature cannot be resolved by the quasi-equilibrium analysis that lead to Equation (3.6).

C. Wall-normal force fields: application to nanofluidic separations and field-flow fractionation

Field-flow fractionation (FFF) is a class of separations where a ‘field’ such as electric field [49,85], magnetic field [86], thermal gradient [87], gravity/centrifugation [83] or a cross-flowing stream [88] is applied perpendicular to the direction of the flow of analyte in a channel/capillary. The analyte is usually transported by a pressure-driven flow. Since the characteristic electrophoretic, magnetophoretic, sedimentation, thermophoretic or convective response of different analytes to the applied field differ (e.g. due to their different charges in solution and/or size), each analyte distributes in a characteristic manner perpendicular to the wall while being transported axially by the flow. The separation principle relies on the fact that, in a pressure-driven flow, analytes that are located closer to the channel/capillary wall on the average move slower than those located closer to the center of the channel.

Recent progress in nanochannel separations have led to both suggestions [41] and experimental realizations [40,89,90] to the effect that electric field-flow fractionation is ‘autogenous’ in nanochannels; a pressure-driven [90,91] (or electrokinetically driven [39,89]) passage of the

sample will automatically lead to separation by the FFF principle and no application of the external normal field is necessary. This is because thin regions ($\sim nm$) of large electric fields ($\sim 10^7$ V/m) in the form of electrical double layers (EDL) exist close to surfaces in contact with the aqueous electrolyte. Only for nanochannels, the EDLs on the two walls occupy a fraction of the channel width significant enough to influence axial analyte motion.

The micrographs in Figure 6 from [89] and [40] show the unique effects of nanochannel electrokinetic transport. Figure 6(a) shows a microfluidic chip interfacing an array of 2000 parallel nanochannels of width 50 nm, depth into the plane of paper of 500 nm, having negatively charged oxidized silicon surface. When a 1:1 mixture of the negatively charged dye Alexa 488 (green under fluorescent illumination) and the neutral dye rhodamine B (red) is transported from right to left through the nanochannel area of the microfluidic chip shown in Figure 6(a) using electroosmotic flow, the dyes separate, with the rhodamine B front lagging the Alexa 488 front, as seen on the fluorescent micrograph (Figure 6(b)) taken 30 s after injection. Pennathur and Santiago [40] found that the relative speed of migration between two differently charged dyes (bodipy and fluorescein) in a 40 nm deep channel differs from that in a 2 μ m deep channel, as evident from the two epifluorescence images in Figure 6(c). The authors of these two works [40,89] also put forth and verified against their experimental observations, models based on the interaction of the transverse electromigration of the charged species with the EOF streamlines; for further details, consult the original works [40,89].

An important characteristic of analyte migration and dispersion in a field-flow fractionation setup arises from the fact that the cross-channel distribution of the analyte can never be uniform, however fast the cross-channel diffusion is, unlike the situation in chromatographic or electrophoretic systems. In other words, unlike situations studied so far in this review, $c \approx \bar{c}$ is an incorrect leading order approximation of the concentration field even in the long time limit, because the competition between diffusive fluxes and migration fluxes resulting from the 'field' in the direction normal to the walls makes the cross-channel distribution nonuniform.

For example, the concentration c in a plane channel of half-width h can be split as $c = f(y)\bar{c} + c'$ where y is a dimensionless cross-channel coordinate (scaled by h) measured from the midplane of the channel, $f(y) = \exp(E(y)/kT) / \overline{\exp(E(y)/kT)}$ is a factor (normalized for convenience such that $\bar{f} = 1$) representing this Boltzmann equilibrium [41] (cf. Table I) and c' is a fluctuation. The bar over a function of y indicates its cross-channel average. On the basis of the above decomposition (or using equivalent approaches), the retention ratio and the dispersivity for a plane channel can be calculated from [4,41,92]:

$$R = \frac{\overline{uf}}{\bar{u}} \quad (3.7a)$$

$$\gamma = \int_0^1 \frac{1}{f(\eta)} \left(\int_0^\eta f(\xi) \left(\frac{u(\xi)}{\bar{u}} - \frac{\overline{uf}}{\bar{u}} \right) d\xi \right)^2 d\eta \quad (3.7b)$$

The decomposition $c = f(y)\bar{c} + c'$ can also be used to generalize the asymptotic reduction approach that lead to Equation (3.3) to field-flow fractionation and obtain Equation (3.7). Equation (3.7a) is also shown on the fifth row of Table I.

Specification of the potential energy function $E(y)$ of the normal field force will give rise to f , R and γ for a particular field-flow fractionation technique. For example, electric field-flow fractionation (studied e.g. in [49]) corresponds to $E(y) = ZeVy/2$ where Z is the valence of the

analyte, V is an applied voltage between the walls and e is the electronic charge, if the mobility of ions under the applied electric field normal to the walls can be calculated with the Nernst Einstein relationship [93].

Autogenous electric FFF of small ions in nanochannels [41] (termed EKSIV [39] when electrokinetic transport is employed) corresponds to choosing the potential energy function $E = Ze\phi$ (cf. Table I) where ϕ is the electrical potential in the electrical double layer (as mentioned in the third column). If the potential on the nanochannel walls is of the same order as the thermal potential kT/e (~ 25.6 mV at $T = 298$ K), the Debye Hückel approximation for $\phi(y)$ in a plane channel can be used [51].

Figure 7 shows the retention ratio in autogenous electric FFF (under pressure-driven flow) calculated using Equation (3.7a) for ions of various signed valences for a channel with a positive wall zeta potential of kT/e , using the Debye-Hückel approximation for $\phi(y)$ (see e.g. [41]). See for example, Figure 3 of [41] for a more detailed graph of the same quantity. At all values of κh , cations are repelled toward the center of the channel and move faster than the anions. For large κh the EDLs are limited to infinitesimal regions close to the walls and most species move through the net-charge-free bulk liquid outside the EDL, regardless of their charge; therefore the velocity of a charged species is nearly identical with that of a neutral ($R \approx 1$). On the other hand for very small values of κh (overlapping EDLs) the electric field becomes too weak to support a significant degree of selective cross-distribution of ions leading again to the same speed of motion for all ions. There is therefore an optimum degree of double layer overlap where the autogenous electric FFF mechanism can be taken advantage of [89, 94]. This information can be used to choose the channel size. Since, the wall is the location of the highest magnitude of electric potential, unlike charges attracted toward the wall are decelerated to a higher degree than the like charges are accelerated.

Inside a nanochannel carrying pressure-driven flow, neutral molecules undergo dispersion by the classical Taylor-Aris dispersion mechanism for pressure-driven flows if streaming potential effects can be neglected. The corresponding Taylor dispersivity γ_P can be used to normalize the dispersivity for ions. Figure 8 shows the dispersivity calculated using Equation (3.7b) and the Debye-Hückel approximation for ions of various signed valences for a channel with a positive wall zeta potential of kT/e . Figure 8 shows that for a positively charged channel, anions which distribute preferentially toward the high-shear regions of the channel undergo more dispersion than cations. Expectedly, the dispersivity approaches the classical Taylor-Aris value for a purely pressure-driven flow both in the limit of thin and thick Debye layers.

Due to the small channel height h , nanochannels offer diffusive equilibration times $O(h^2/D)$ that are about six order of magnitudes shorter than in microchannels. The corresponding zone broadening, as measured by the plate height is also smaller ($\sim \mu\text{m}$) compared to microchannels ($\sim \text{mm}$) [95]. However, zone broadening considerations are still relevant due to technological limitations in simultaneously detecting small sample volumes and low concentrations [27]. Quantitative knowledge of the dispersion coefficients in a nanochannel can therefore be useful.

In emerging nanofluidic applications [94,95] e.g. employing nanochannel arrays and classical colloidal transport, an important consideration is the steric exclusion of large biomolecules from the region near nanochannel walls. While investigating colloidal transport in circular pores, ref. [48] derived a version of Equation (3.7) applicable to large spherical solutes in circular pores of comparable radius. In general, the role of surface charge and adsorption of analyte are also more significant in nanochannel separations than in microchannels owing to the larger surface to volume ratio [94,96]. As scale of separation devices get smaller, non-continuum effects on the ionic distribution and fluid flow [97,98] may assume significance [27] but are beyond the scope of this review.

IV. CONCLUSIONS

The role of dispersion in reducing the efficiency of microfluidic separations was reviewed. The metrics such as retention ratio and plate height for theoretical as well as operational characterization of analyte migration and dispersion were defined and discussed to contextualize the importance of characterizing dispersion. Factors affecting analyte migration and dispersion in presence of three kinds of physical processes in the separation channel, namely inhomogeneous flow fields, interactions of analyte with the wall and force fields normal to the wall were reviewed. Selected applications of analyte migration and dispersion calculations in characterizing the separation efficiency of capillary electrophoresis, capillary electrochromatography, liquid chromatography, shear-driven-chromatography and field-flow fractionation in microchannels as well as nanochannels has been highlighted from the literature. While discussing these applications, the prediction of dispersion in established (*e.g.* liquid chromatography or capillary electrochromatography under pressure and electrokinetic actuation, respectively), relatively innovative (*e.g.* autogenous field-flow fractionation in nanochannels with no external field, capillary electrophoresis with surface patterned microchannels) and problematic (*e.g.* capillary electrophoresis under conditions when analyte adsorbs non-specifically to the walls) circumstances in such separations have been considered. Quantifying dispersion through a flexible theoretical/computational framework applicable at once to several physicochemical conditions (*e.g.* fast/slow adsorption, axially variable/axially uniform flows) as espoused in this article can help us identify design and control strategies toward faster and more efficient microfluidic and nanofluidic separations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1. Dispersion of a fluorescent dye in a $250\ \mu\text{m} \times 70\ \mu\text{m}$ rectangular microchannel under pressure-driven flow. See [9], used here with permission of the authors.

FIG. 2.

Schematic of a microfluidic chip with separation and injection channels crossing each other. The distance L between injection and detection ports on the separation channel is typically of the order of several centimeters and the channel width is typically of the order of several tens of microns.

FIG. 3.

Chromatograms showing different composition of white (top) and red (bottom) wines [17]. An optical detection method was used; the number of *mAU* (milli-absorbance units) for each peak is proportional to the concentration of each constituent (1. Prothocatechuic acid; 2. Gallic acid; 3. (+)-Catechin; 4. (-)-Epicatechin; 5. *trans*-resveratrol; 6. Rutin). Used with permission of the authors.

FIG. 4.

Effective dispersion coefficient (D_{eff}) vs. time (t) for electroosmotic flow with mean velocity $\bar{u}$ under an axially varying zeta potential of wavelength Λ in a square channel of side $2b$ calculated from three-dimensional numerical simulation (solid line) and simulation of one-dimensional asymptotic equations (circular symbols) [62]. Parameters are, top panel: $\Lambda = L/5 = 51.2b$, center panel: $\Lambda = L/10 = 25.6b$ and lower panel: $\Lambda = L/25 = 10.24b$ respectively. The amplitude $\Delta\zeta$ of the zeta potential variation was 75% of the axial average $\langle\zeta\rangle$ of the zeta potential. The Peclet number ($2b\bar{u}/D$) was 5. See [62].

FIG. 5.

Effective Dispersion Coefficient (D_{eff}) at the detection time $t = 16b/\bar{u}$ vs. adsorption rate constant (λKPe) for a neutral analyte suffering wall adsorption according to a linear kinetic law while being transported by an electroosmotic flow in a square microchannel of side $2b$ calculated from 3-D numerical simulation, 1-D numerical simulation and chromatographic theory of analyte transport. The dimensionless adsorption rate constant $\lambda = 2k_d b/\bar{u}$ is varied keeping the dimensionless equilibrium constant $K = k_a/(2k_d b) = 1$ and $Pe = \bar{u}(2b)/D = 10$ fixed. Results from three-dimensional numerical simulation are represented by asterisks and results from simulation of the asymptotically reduced model (Equation (3.3)) are represented by circular symbols. The solid line is the effective dispersion coefficient calculated from the theory of chromatography. Here, $Pe = \bar{u}(2b)/D = 10$ and the length of simulation domain $L = 40b$. See [62].

FIG. 6.

Figure 6(a), adapted from [89], shows the setup used in their electrokinetic transport experiments of Garcia *et al.*. Figure 6(b), adapted from [89], is a fluorescence micrograph of the nanochannel region of Figure 6(a), showing the separation of the negatively charged dye Alexa 488 (green) from the neutral dye Rhodamine B (red), under an electroosmotic flow. Figure 6(c), adapted from [40], shows epifluorescence images of the transport of the charged dyes bodipy and fluorescein in a 2 μm deep microchannel (top pane), and in a 40 nm deep nanochannel (bottom pane), as observed by Pennathur and Santiago. Figure 6(b)–6(c) demonstrate the autogenous field-flow fractionation effect [41] of the electrical double layers within a nanochannel. Figures 6(a) and 6(b) are reproduced by permission of The Royal Society of Chemistry. Figure 6(c) is reproduced by permission of the American Chemical Society.

FIG. 7.

The retention ratio of ions in autogenous electric field-flow fractionation under pressure-driven flow in a nanochannel of width $2h$ with a positive zeta potential of kT/e on the walls as a function of the scaled Debye Hückel parameter κh . Z is the valence of the ion. See also [41].

FIG. 8.

The dispersivity of ions γ normalized by the Taylor dispersivity $\gamma_p = \frac{2}{105}$ in autogenous electric field-flow fractionation under pressure-driven flow in a nanochannel of width $2h$ with a positive zeta potential of kT/e on the walls as a function of the scaled Debye Hückel parameter κh . Z is the valence of the ion. See also [41].

TABLE I

Migration speed in microfluidic and nanofluidic separations. The acronyms GC, LC, GPC and EKSIV stand for gas chromatography, liquid chromatography, gel permeation chromatography and Electrokinetic Separation by Ion Valence respectively.

Separation Method	Retention ratio (R)	Remarks	Reference
GC/LC/GPC	$\frac{1}{1+k}$	$R \leq 1$	[42–44]
Capillary electrophoresis	$\frac{\mu_{ep} + \mu_{eo}}{\mu_{eo}}$		[45]
Capillary electrochromatography	$\frac{\mu_{ep} + \mu_{eo}}{\mu_{eo}(1+k)}$		[45]
Hydrodynamic chromatography	$K_c(\lambda)$	$\lambda < 1, K_c > 1$	[4,46,47]
Field-flow fractionation	$\frac{F \exp(-E(y, z)/kT)}{\exp(-E(y, z)/kT)}$	Case $E = Z\phi e$ gives EKSIV principle [39]	[39,41,48,49]

TABLE II

The dispersivity γ for a pressure-driven, wall-shear-driven and finite Debye-layer low zeta potential electroosmotic flow in a plane channel with half-height h , y being the cross-channel coordinate measured from the mid-plane of the channel. In case of the shear-driven flow, the top wall at $y = h$ is moving. The electroosmotic flow profile is calculated based on the Debye-Hückel approximation [51], the Debye Hückel parameter κ is defined in Equation (3.2).

Flow pattern	Flow shape $\left(\frac{u}{a}\right)$	γ
Pressure-driven	$\frac{3}{2} [1-(y/h)^2]$	$\frac{2}{105}$
Wall-shear driven (Couette)	$[(y/h) + 1]$	$\frac{2}{15}$
Electroosmotic [66]	$\frac{\kappa h \cosh \kappa y}{\sinh \kappa h}$	$\frac{(2\kappa^2 h^2 + 12) \sinh^2(\kappa h) - 9\kappa h \cosh(\kappa h) \sinh(\kappa h) - 3\kappa^2 h^2}{6\kappa^2 h^2 [\sinh(\kappa h) - \kappa h \cosh(\kappa h)]^2}$

TABLE III

The coefficients δ , β and γ (*cf.* Equations (3.1),(3.3),(3.4),(3.5) and (3.6)) calculated for an axially homogeneous and purely pressure-driven flow in a plane channel (gap between parallel plates) and in channels with circular, square and trapezoidal cross-section. These coefficients can be obtained by solving Equations (3.4) and (3.5) for the functions ψ and χ . The trapezoid in the last column is isosceles where the distance between the parallel sides equals half the sum of the parallel sides. The reference length-scale h , for the purposes of this table, is the diameter for the circle and the gap between the parallel sides for the other geometrical shapes.

Channel Type	Plane	Circular	Square	Trapezoidal
δ	$\frac{1}{12}$	$\frac{1}{32}$	$\frac{1}{24}$	0.113
β	$\frac{1}{60}$	$\frac{1}{96}$	0.0155	0.052
γ	$\frac{1}{210}$	$\frac{1}{192}$	0.0084	0.0329

TABLE IV

Calculation of the mobile phase band broadening factor C in plane channels and cylindrical channels for open channel modes of chromatography. CEC stands for Capillary Electrochromatography, LC stands for Liquid Chromatography, GC for gas chromatography, SDC stands for shear-driven chromatography and ACEO stands for the AC-electroosmosis based cyclic separation technique described in [65]. In order to use these expressions in Equation (3.6), h should be taken as the width for the plane channel and radius for the cylindrical channel.

Mode	Flow	Cylindrical capillary	Plane channel	Reference
CEC	EOF (Uniform)	$\frac{k^2}{32(1+k)^2}$	$\frac{k^2}{12(1+k)^2}$	[84]
LC/GC	Pressure-driven Flow	$\frac{11k^2 + 6k + 1}{48(k+1)^2}$	$\frac{51k^2 + 18k + 2}{420(k+1)^2}$	[42]
SDC/ACEO	Couette Flow	Not applicable	$\frac{32k^2 + 14k + 2}{60(k+1)^2}$	[64]