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Estimating Stream Broadening in Free-Flow Electrophoretic Systems based on the Method-of-Moments Formulation

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Abstract

The resolving power of free-flow electrophoretic assays is often limited by the broadening of analyte streams as they migrate through the separation chamber. While molecular diffusion and flow hydrodynamics inherently contribute to such dispersion, non-idealities such as Joule heating and unwanted pressure-driven cross flows among others can also significantly modify these contributions. In this chapter, we describe a theoretical approach to estimating stream broadening in free-flow electrophoretic systems under steady-state conditions based on the method-of-moments formulation. This methodology allows the determination of the spatial moments of solute concentration, thereby yielding measures for the mean position and spatial variance of the analyte stream.

Keywords

electrodynamic dispersion; free-flow electrophoresis; free-flow isoelectric focusing; hydrodynamic dispersion; method-of-moments

1. Introduction

Continuous separation of a target sample fraction from a complex mixture is desirable in several applications that either require an enrichment of the analyte molecules in the specimen for its further downstream processing or a reduction in interference from the matrix species during analyte detection. Free-flow electrophoretic (FFE) techniques offer a useful approach in this regard enabling the extraction of analytes based on their electrophoretic mobilities or isoelectric points, and are applicable to a wide range of chemical and biological samples [1]. Moreover, the use of relatively gentle operating conditions in these techniques combined with their high throughput make them particularly valuable tools for simplifying the sample feed to an electrophoretic or chromatographic system. Free-flow electrophoretic assays typically rely on the continuous pressure-driven flow of a sample stream through a separation chamber in the presence of an electric field applied perpendicular to this flow direction. In this situation, analyte molecules are deflected from their original path based on their electrophoretic mobilities causing them to exit the separation chamber at different lateral positions. The resolution of the separation process

consequently, is determined by the differences in the electrophoretic transport rates or isoelectric points of the solute species as well as the amount of band dispersion they undergo during their transit through the analysis column [2].

Stream broadening in free-flow electrophoretic systems primarily occur due to molecular diffusion orthogonal to the flow direction as well as a variation in the transit time for the analyte molecules when travelling along the different streamlines in the system. While the first contribution to stream broadening is relatively simple to estimate, the latter one is determined by a complex interplay between the local streamline velocity and analyte diffusion across these streamlines [3]. It has been established that the latter effect is particularly prominent at moderate to large transverse electric fields and can originate for a variety of reasons. In this chapter, we present a general approach to estimating the mean position and spatial variance of analyte streams as they migrate through a free-flow electrophoresis column. This approach is based on the method-of-moments formulation and can assess the resolving power of FFE methods under ideal conditions over a wide range of time and length scales. In addition, the noted approach allows the determination of leading order effects of common non-idealities such as Joule heating and unwanted pressure-driven cross-flows among others on the resolution of FFE separations, thereby providing insights for developing strategies to minimize them.

2. Materials

This chapter discusses theoretical results on stream broadening in free-flow electrophoretic systems which are applicable to a broad range of separation conditions, e.g., buffer compositions, electrophoretic mobilities, electric fields, pressure-driven flow rates, channel dimensions, etc. However, our theoretical models require an estimate for the magnitudes of these quantities as well as their dependence on various parameters such as temperature, buffer pH, etc., in order to formulate the governing mathematical equations. In addition, we often need to assume certain properties about the separation buffer (e.g., Newtonian fluid) and analyte species (e.g., isotropic diffusion) in order to simplify our mathematical treatment of the problem which will be noted at appropriate points in this chapter. While many of these assumptions/approximations may not be strictly valid for a specific experimental condition, the results included here can be still useful for broadly assessing the separation performance of FFE assays, thereby enabling the preliminary optimization of their operating conditions.

3. Methods

3.1 Mathematical Formulation

To evaluate stream dispersion in a FFE system, we consider the flow of an analyte stream between two parallel plates (*see* Note 1) separated by a distance d (*see* Fig. 1) under the influence of a pressure-driven flow in the axial direction (along the z -coordinate) and an electric field (E) applied across the width of the separation chamber (along the x -coordinate). In order to simplify our mathematical analysis, we assume the locations of the

parallel plates to be $y = \pm d/2$ yielding a pressure-driven velocity profile $u_z = (3\bar{U}_z/2)(1 - 4y^2/d^2)$ with $\bar{U}_z$ being the spatially averaged value of u_z .

The advection-diffusion equation governing the concentration of the analyte species (C) under steady state in this situation may be written as (see Note 2)

$$\frac{\partial(u_x C)}{\partial x} + u_z \frac{\partial C}{\partial z} = D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial z^2} \right) + \frac{\partial}{\partial y} \left(D \frac{\partial C}{\partial y} \right) \quad (1)$$

where u_x and D refer to the analyte migration velocity along the x -coordinate and analyte diffusion coefficient in the system, respectively. Notice that the quantity u_x in this formulation may vary with the x - and y -coordinates as in a free-flow isoelectric focusing assay with an unwanted pressure-driven cross-flow while the diffusion coefficient D may only be a function of y say due to a temperature variation across the channel depth arising from Joule heating effects. Upon normalizing all length scales with respect to d , i.e., $x^* = x/d$, $y^* = y/d$, $z^* = z/d$, the sample concentration by its inlet value (C_0), i.e., $C^* = C/C_0$, and the diffusion coefficient by its magnitude at a reference point, e.g., channel walls ($y^* = \pm 1/2$), Eq. (1) may be reduced to the dimensionless form

$$Pe_x \frac{\partial(u_x^* C^*)}{\partial x^*} + \frac{3}{2} Pe_z (1 - 4y^{*2}) \frac{\partial C^*}{\partial z^*} = \frac{D}{D_0} \left(\frac{\partial^2 C^*}{\partial x^{*2}} + \frac{\partial^2 C^*}{\partial z^{*2}} \right) + \frac{\partial}{\partial y^*} \left(\frac{D}{D_0} \frac{\partial C^*}{\partial y^*} \right) \quad (2)$$

where $u_x^* = u_x/\bar{U}_x$. The quantities $Pe_x = \bar{U}_x d/D_0$ and $Pe_z = \bar{U}_z d/D_0$ here denote the Péclet numbers in the x - and z -directions, respectively, yielding measures of advective transport relative to diffusion along the width and length of the separation compartment. The analyte concentration in this system is subjected to the boundary conditions $C^*/y^* = 0$ at $y^* = \pm 1/2$, $C^*/x^* = 0$ as $x^* \rightarrow \pm \infty$ and $C^* = 1$ at $z^* = 0, -b/2d \leq x^* \leq b/2d$ where b denotes the width of the sample stream at the inlet location ($z^* = 0$). In addition to these constraints, the amount of analyte flowing per unit time through any x - y plane is a constant M in our analysis which can be equated to the integral $(3C_0 \bar{U}_z d^2/2) \int_{-1/2}^{1/2} \int_{-\infty}^{\infty} (1 - 4y^{*2}) C^* dx^* dy^*$ to satisfy the material balance in the system. Now multiplying equation (2) with x^{*p} followed by integrating it along the x^* -coordinate from $-\infty$ to ∞ , it is possible to show that [4]

$$\frac{3}{2} Pe_z (1 - 4y^{*2}) \frac{\partial \phi_p}{\partial z^*} - p Pe_x \int_{-\infty}^{\infty} x^{*p-1} u_x^* C^* dx^* = p(p-1) \frac{D}{D_0} \phi_{p-2} + \frac{\partial}{\partial y^*} \left(\frac{D}{D_0} \frac{\partial \phi_p}{\partial y^*} \right) + \frac{D}{D_0} \frac{\partial^2 \phi_p}{\partial z^{*2}}$$

$$\text{Boundary conditions: } \left. \frac{\partial \phi_p}{\partial y^*} \right|_{y^* = \pm 1/2} = 0 ; \int_{-1/2}^{1/2} (1 - 4y^{*2}) \phi_0 dy^* = \frac{2M}{3C_0 \bar{U}_z d^2} = \frac{2\delta}{3} \quad (3)$$

where $\phi_p = \int_{-\infty}^{\infty} x^{*p} C^* dx^*$. Further integrating Eq. (3) along the y^* -coordinate over the region between the parallel plates and defining $m_p = \int_{-1/2}^{1/2} \phi_p dy^*$ one can obtain

$$\begin{aligned} & \frac{3}{2} Pe_z \int_{-1/2}^{1/2} \left[(1 - 4y^{*2}) \frac{\partial \phi_p}{\partial z^*} \right] dy^* - p Pe_x \int_{-1/2}^{1/2} \int_{-\infty}^{\infty} x^{*p-1} u_x^* C^* dx^* dy^* \quad (4) \\ & = p(p-1) \frac{D}{D_0} m_{p-2} + \frac{D}{D_0} \frac{\partial^2 m_p}{\partial z^{*2}} \end{aligned}$$

with $m_0|_{z^*=0} = \delta$, $m_1|_{z^*=0} = 0$ and $m_2|_{z^*=0} = \delta^3/12$ (see Notes 3 and 4). Notice that the quantity m_p in this formulation represents the p^{th} moment of C^* in any x - y plane with m_1 representing the normalized x^* -position of the center of mass for the analyte stream and the quantity $m_2/m_0 - m_1^2/m_0^2$ equaling its normalized spatial variance along the x -axis.

3.2 Hydrodynamic dispersion in free-flow zone electrophoresis

In an ideal free-flow zone electrophoresis (FFZE) assay, the transverse analyte velocity can be simply written as $u_x = \mu E$ where μ refers to the net electrokinetic mobility (algebraic sum of the electrophoretic and electroosmotic mobilities) of the analyte species under the operating conditions. In addition, the diffusion coefficient D may be assumed to be uniform at all locations due to the isothermal nature of the system, i.e., $D = D_0$. In this situation, the equations governing ϕ_p and m_p reduce to the forms

$$\frac{3}{2} Pe_z (1 - 4y^{*2}) \frac{\partial \phi_p}{\partial z^*} - p Pe_x \phi_{p-1} = p(p-1) \phi_{p-2} + \frac{\partial^2 \phi_p}{\partial y^{*2}} + \frac{\partial^2 \phi_p}{\partial z^{*2}} \quad (5)$$

$$\frac{3}{2} Pe_z \int_{-1/2}^{1/2} \left[(1 - 4y^{*2}) \frac{\partial \phi_p}{\partial z^*} \right] dy^* - p Pe_x m_{p-1} = p(p-1) m_{p-2} + \frac{d^2 m_p}{dz^{*2}} \quad (6)$$

Moreover, the integral boundary condition conserving the amount of analyte flowing per unit time through any x - y plane suggests that ϕ_0 , and hence m_0 , cannot be a function of z^* , yielding the solutions $\phi_0 = m_0 = \delta$. Now upon substituting these values into the equation governing ϕ_1 , i.e., Eq. (5) with $p = 1$, it is possible to show that

$$\phi_1 = \frac{Pe_x \delta}{Pe_z} z^* + \frac{Pe_x \delta}{4} (y^{*2} - 2y^{*4}) - \frac{7Pe_x \delta}{480} \quad \text{and} \quad m_1 = \frac{Pe_x \delta}{Pe_z} z^* \quad (7)$$

Further substituting these expressions in Eq. (5) for $p = 2$, one can obtain

$$\phi_2 = \frac{Pe_x^2 \delta}{Pe_z^2} z^{*2} + \left[\frac{Pe_x^2 \delta}{2Pe_z} (y^{*2} - 2y^{*4}) + \frac{2\delta}{Pe_z} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) - \frac{11Pe_x \delta}{560Pe_z} \right] z^* + g(y^*)$$

$$\text{where } g(y^*) = \frac{Pe_x^2 \delta}{1612800} (172800y^{*8} - 188160y^{*6} + 49440y^{*4} - 240y^{*2} - 253) \quad (8)$$

$$- \frac{\delta}{240} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) (240y^{*4} - 120y^{*2} + 7) + \frac{\delta^3}{12}$$

Integrating Eq. (8) across the separation gap between the parallel plates we get

$$m_2 = \frac{Pe_x^2 \delta}{Pe_z^2} z^{*2} + \left[\frac{2\delta}{Pe_z} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) + \frac{Pe_x^2}{105Pe_z} \right] z^* + \frac{\delta^3}{12} \quad (9)$$

Finally, the spatial variance of the sample stream (σ^2) in an FFZE system may be evaluated as

$$\frac{\sigma^2}{d^2} = \frac{m_2}{m_0} - \frac{m_1^2}{m_0^2} = \left[\frac{2}{Pe_z} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) + \frac{Pe_x^2}{105Pe_z} \right] z^* + \frac{\delta^2}{12} \quad (10)$$

which shows that this quantity grows linearly with the axial position (z -coordinate) in the separation chamber. The factor multiplying z^* in this equation is a measure of the effective dispersion coefficient for the analyte zone and the term $\delta^2/12$ represents the contribution from the injection width of the sample stream. Because the normalized axial position z^* scales linearly with the residence time (t) of the analyte molecules in the FFZE compartment, it is then possible to express $\sigma^2 = 2Kt + b^2/12$ yielding a Taylor-Aris dispersion coefficient (K) [3–5]

$$K = D \left[1 + \left(\frac{Pe_x}{Pe_z} \right)^2 + \frac{1}{210} Pe_x^2 \right] \quad (11)$$

Eq. (11) shows that the overall broadening of a sample stream during its transit through an FFZE chamber occurs as a result of molecular diffusion of the analyte species orthogonal to their flow direction as well as hydrodynamic dispersion stemming from a variation in the pressure-driven flow velocity across the gap between the parallel plates. The first contribution to this broadening process is captured by the $D[1 + (Pe_x/Pe_z)^2]$ term in which the $D(Pe_x/Pe_z)^2$ component (orientation factor) arises due to the fact that σ is measured along the x -axis while the sample stream makes an angle $\theta = \cot^{-1}(Pe_x/Pe_z)$ with respect to

this line. The $D(Pe_x^2/210)$ term in Eq. (11) quantitates the hydrodynamic dispersion component, and interestingly has the same numerical coefficient, i.e., $1/210$, as expected during the advection of analyte bands by an axial pressure-gradient. However, it must be noted that while the Péclet number multiplying this coefficient is evaluated based on the lateral electrophoretic velocity of the analyte species in an FFZE assay, this dimensionless group in the other case depends on the longitudinal pressure-driven flow speed in the system. It is important to point out that the expression derived for quantitating the hydrodynamic dispersion component in the current work is identical to that empirically proposed by Fonslow and Bowser in a recent publication based on their experimental observations [6]. In this situation, the mathematical analysis presented here offers a rigorous justification for the agreement between Fonslow and Bowser's proposed theory and experimental results.

The normalized expression in Eq. (11) although describes stream dispersion in FFZE separations in terms of the smallest set of variables, it does not highlight the dependence of σ^2 on the different operating parameters very well. To bring out these relationships, the stream variance along the x -coordinate at the channel exit may be rewritten in its dimensional form as

$$\sigma^2 = \underbrace{\frac{2D_0L}{\bar{U}_z}}_{\text{diffusive broadening}} + \underbrace{\frac{2\mu^2E^2D_0L}{\bar{U}_z^3}}_{\text{orientation factor}} + \underbrace{\frac{1}{105} \frac{\mu^2E^2d^2L}{\bar{U}_zD_0}}_{\text{hydrodynamic broadening}} + \underbrace{\frac{b^2}{12}}_{\text{injection variance}} \quad (12)$$

where L denotes the length of the FFZE channel. In order to emphasize the relative importance of the various contributions to stream broadening in Eq. (12), we have plotted them individually in Fig. 2(a) as a function of the pressure-driven velocity, $\bar{U}_z$. The values for the other operating conditions in this figure were chosen to be $E = 500$ V/cm, $d = 20$ μm , $D_0 = 8 \times 10^{-6}$ cm^2/s , $\mu = 5 \times 10^{-4}$ cm^2/Vs , $L = 2.5$ cm and $b = 100$ μm that are commonly employed in microfluidic FFZE fractionations. As may be seen from the figure, the dominant contribution to σ^2 under the chosen conditions comes from hydrodynamic dispersion of the analyte stream for values of $\bar{U}_z$ in the range of 0.05–5 cm/s. What is also interesting to note is that this component diminishes with an increase in the value of $\bar{U}_z$ following a reverse trend to that observed in pressure-driven band separations. This result arises from the fact that the residence time of the analyte molecules in the separation channel is reduced with an increase in the pressure-driven velocity for a fixed value of E . Alternatively, if one compares the different contributions to σ^2 for a fixed migration distance of the analyte zone in the lateral direction ($S = \mu EL/\bar{U}_z$), Eq. (12) translates to

$$\sigma^2 = \frac{2D_0L}{\bar{U}_z} + \frac{2S^2D_0}{\bar{U}_zL} + \frac{1}{105} \frac{S^2d^2\bar{U}_z}{D_0L} + \frac{b^2}{12} = \underbrace{\frac{b^2}{12}}_A + \underbrace{\left(2D_0L + \frac{2S^2D_0}{L}\right)}_B \frac{1}{\bar{U}_z} + \underbrace{\frac{1}{105} \frac{S^2d^2}{D_0L}}_C \bar{U}_z \quad (13)$$

yielding an expression similar to the van Deemter equation [7] commonly used for describing sample dispersion in band separations. In Fig. 2(b), we have compared the different additive terms in Eq. (13) for the same set of operating parameters used in Fig. 2(a) except for replacing the condition $E = 500$ V/cm with $S = 5$ mm. In this comparison, the hydrodynamic dispersion component is seen to dominate only for $\bar{U} \gg 3$ mm/s with the diffusive broadening contribution dictating σ^2 in the opposite limit. The A , B and C terms defined in Eq. (13) were further evaluated in our work by fitting experimental measurements of the stream variance reported by Fonslow and Bowser (data taken from Fig. 6(a) in reference [6]) to a curve of the form $\sigma^2 = A + B/\bar{U}_z + C\bar{U}_z$ (see Fig. 3(a)). In Fig 3(b), we have then plotted the estimates of these quantities as a function of the parameter S in the system. The figure shows the C term in this mathematical description to only vary with S indicating the contribution to stream variance from the orientation factor to be negligible in Fonslow and Bowers work. Subsequently, the estimates for the C term were fitted to an equation of the form $C = kS^2$ to yield $A = 4.9 \times 10^{-5} \text{ cm}^2$, $B = 8.3 \times 10^{-5} \text{ cm}^3/\text{s}$ and $k = 1.4 \times 10^{-2} \text{ s/cm}$. These values correspond to an injection stream width (b) and analyte diffusion coefficient (D_0) of $242 \text{ }\mu\text{m}$ and $1.67 \times 10^{-5} \text{ cm}^2/\text{s}$, respectively. The estimate for the quantity k on the other hand yielded a factor $1/7$, instead of $1/105$, for the numerical coefficient in the hydrodynamic dispersion term suggesting that this contribution exceeded our theoretical prediction by a factor of 15 in Fonslow and Bowser's device likely due to non-idealities in the system.

3.3 Electrodynamic dispersion in free-flow zone electrophoresis

Electrodynamic dispersion of sample streams in FFZE assays can originate due to partial or complete blockage of electroosmotic flow (EOF) across the channel width by the sidewalls of the conduit. Such blockage of EOF then generates a pressure-driven backflow in the transverse direction for maintaining a flow balance in the system leading to additional stream broadening. In this situation, the analyte velocity in the transverse direction may be expressed as $u_x = \mu E \left[1 - (3\alpha/2)(1 - 4y^2/d^2) \right]$ in regions far away from the channel sidewalls [8]. Notice that the spatially averaged value of u_x in this description equals $\mu E(1 - \alpha)$ which determines the lateral migration distance for the analyte stream at the channel exit. Moreover, the diffusion coefficient D may again be assumed to be the uniform at all locations as the system is isothermal, i.e., $D = D_0$. These considerations yield equations governing ϕ_p and m_p given by

$$\begin{aligned} & \frac{3}{2}Pe_z(1 - 4y^{*2})\frac{\partial\phi_p}{\partial z^*} - \frac{pPe_x}{(1 - \alpha)}\left[1 - \frac{3}{2}\alpha(1 - 4y^{*2})\right]\phi_{p-1} \quad (14) \\ & = p(p-1)\phi_{p-2} + \frac{\partial^2\phi_p}{\partial y^{*2}} + \frac{\partial^2\phi_p}{\partial z^{*2}} \end{aligned}$$

$$\begin{aligned} & \frac{3}{2}Pe_z \int_{-1/2}^{1/2} \left[(1 - 4y^{*2}) \frac{\partial \phi_p}{\partial z^*} \right] dy^* - \frac{pPe_x}{(1-\alpha)} \int_{-1/2}^{1/2} \left[1 - \frac{3}{2}\alpha(1 - 4y^{*2}) \right] \phi_{p-1} dy^* \quad (15) \\ & = p(p-1)m_{p-2} + \frac{d^2 m_p}{dz^{*2}} \end{aligned}$$

The integral boundary condition conserving the amount of analyte flowing per unit time through any x - y plane again suggests that ϕ_0 , and hence m_0 , cannot be a function of z^* , yielding the solutions $\phi_0 = m_0 = \delta$. Moreover, upon substituting these values into the equation governing ϕ_1 , i.e., Eq. (14) with $p = 1$, it is possible to show that

$$\phi_1 = \frac{Pe_x \delta}{Pe_z} z^* + \frac{Pe_x \delta}{480(1-\alpha)} (120y^{*2} - 240y^{*4} - 7) \quad \text{and} \quad m_1 = \frac{Pe_x \delta}{Pe_z} z^* \quad (16)$$

Further substituting these expressions in Eq. (14) for $p = 2$, one can obtain

$$\phi_2 = \frac{Pe_x^2 \delta}{Pe_z^2} z^{*2} + \left[\frac{Pe_x^2 \delta}{2Pe_z(1-\alpha)} \left((y^{*2} - 2y^{*4}) - \frac{(33-49\alpha)}{840(1-\alpha)} \right) + \frac{2\delta}{Pe_z} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) \right] z^* + g(y^*)$$

$$\begin{aligned} \text{where } g(y^*) = & \frac{Pe_x^2 \delta}{1612800(1-\alpha)^2} (172800y^{*8} - 188160y^{*6} + 49440y^{*4} - 240y^{*2} - 253) \\ & - \frac{\delta}{240} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) (240y^{*4} - 120y^{*2} + 7) + \frac{\delta^3}{12} \end{aligned}$$

(17)

Integrating Eq. (17) across the separation gap between the parallel plates we get

$$m_2 = \frac{Pe_x^2 \delta}{Pe_z^2} z^{*2} + \left[\frac{2\delta}{Pe_z} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) + \frac{Pe_x^2 \delta}{105Pe_z(1-\alpha)^2} \right] z^* + \frac{\delta^3}{12} \quad (18)$$

Finally, the spatial variance of the sample stream (σ^2) in an FFZE system may be evaluated as

$$\frac{\sigma^2}{d^2} = \frac{m_2}{m_0} - \frac{m_1^2}{m_0^2} = \left[\frac{2}{Pe_z} \left(1 + \frac{Pe_x^2}{Pe_z^2} \right) + \frac{Pe_x^2}{105Pe_z(1-\alpha)^2} \right] z^* + \frac{\delta^2}{12} \quad (19)$$

which shows that this quantity grows linearly with the axial position (z^* -coordinate) in the separation chamber. The factor multiplying z^* in this equation is a measure of the effective dispersion coefficient for the analyte zone while the term $\delta^2/12$ represents the contribution from the injection width to the overall sample variance. Because the normalized axial position z^* scales linearly with the residence time ($t = z/\bar{U}_z$) of the analyte molecules in the FFZE compartment, it is then possible to express $\sigma^2 = 2Kt + b^2/12$ yielding a Taylor-Aris dispersion coefficient (K)

$$K = D \left[1 + \left(\frac{Pe_x}{Pe_z} \right)^2 + \frac{1}{210} \frac{Pe_x^2}{(1 - \alpha)^2} \right] \quad (20)$$

Eq. (20) shows that the overall broadening of a sample stream during its transit through an FFZE chamber occurs as a result of molecular diffusion of the analyte species orthogonal to its flow direction as well as hydrodynamic dispersion stemming from a variation in the analyte velocity in the x and z -directions across the gap between the parallel plates. The first contribution to this broadening process is captured by the $D_0[1 + (Pe_x/Pe_z)^2]$ term in which the $D_0(Pe_x/Pe_z)^2$ component (orientation factor) simply arises due to the fact that σ is measured along the x -axis while the sample stream makes an angle $\theta = \cot^{-1}(Pe_x/Pe_z)$ with respect to this line. Notice that for a given transverse electric field (E) and axial pressure-driven flow speed ($\bar{U}_z$), the orientation factor varies as $D_0[\mu E(1 - \alpha)/\bar{U}_z]^2$ whereas for a given lateral migration distance $S = \mu E(1 - \alpha)L/\bar{U}_z$ of the analyte stream, this factor equals

$D_0(S/L)^2$ and is independent of α . The other term in Eq. (20), i.e., $D_0[(1/210)\{Pe_x^2/(1 - \alpha)^2\}]$, quantitates the hydrodynamic dispersion component in the system and differs by a factor of $1/(1 - \alpha)^2$ from the case of no transverse pressure-driven flow. This comparison however, is only valid for a fixed Pe_x or lateral migration distance (S) of the sample zone. For a given transverse electric field in the FFZE assay, the hydrodynamic dispersion component reduces to $D_0(\mu Ed/D_0)^2/210$ which is identical to the case of no transverse pressure-driven flow. This is a very interesting result which says that although the pressure-driven cross-flow modifies the lateral migration distance for an analyte zone, it does not alter its spatial variance for a given transverse electric field in the assay. In other words, the pressure-driven cross-flow does not lead to any additional dispersion in the system. In fact, if one solves the transport equations relevant to our problem in the absence of a transverse electric field but with a non-zero pressure-driven cross-flow, the Taylor-Aris dispersion coefficient can be shown to be given by $K = D_0[1 + (\bar{U}_x/\bar{U}_z)^2]$ where $\bar{U}_x$ and $\bar{U}_z$ correspond to the spatially averaged

pressure-driven velocities in the x and z -directions, respectively. This expression again does not have any contribution from hydrodynamic dispersion, consistent with our analysis of an FFZE process with a lateral pressure-driven backflow. The lack of hydrodynamic dispersion in the presence of both axial and transverse pressure-gradients is a consequence of the fact that although solute molecules traveling along the channel center ($y^* = 0$) in these systems experience a higher lateral velocity, their transit time in the channel is shorter as they migrate along the faster moving streamlines in the axial direction. Consequently, the lateral

distance drifted by these entities is identical to that travelled by molecules residing close to the channel walls at $y^* = \pm 1/2$.

For a given lateral migration distance in an FFZE device however, pressure-driven cross-flows can significantly influence the performance of the assay irrespective of whether they arise due to blockage of EOF by the channel sidewalls or as a result of unwanted pressure-gradients across the width of the separation chamber. If one analyzes the expression describing the variance of a sample stream under these conditions by rewriting Eq. (19) as

$$\sigma^2 = \underbrace{\frac{b^2}{12}}_A + \underbrace{\left(2D_0L + \frac{2S^2D_0}{L}\right)}_B \frac{1}{\bar{U}_z} + \underbrace{\frac{1}{105} \frac{S^2d^2}{(1-\alpha)^2D_0L}}_C \bar{U}_z \quad (21)$$

the C term in it can be significantly underestimated when any pressure-driven backflow is ignored. The discrepancy noticed in the coefficient multiplying the ratio S^2d^2/D_0L as estimated from experiments and theory in Fonslow and Bowser's work [6], for example, may at least in part be explained by the presence of a transverse pressure-gradient in their system. Moreover, the greater dispersion caused by such a backflow for a fixed magnitude of S as predicted by Eq. (21) shifts the optimum axial flow velocity ($\bar{U}_{z, opt}$) at which the stream variance is minimized to lower values as

$$\bar{U}_{z, opt} = \sqrt{105 \left(2D_0L + \frac{2S^2D_0}{L}\right) \frac{D_0L(1-\alpha)^2}{S^2d^2}} \quad (22)$$

The minimum stream variance realized under these conditions is also seen to increase with α as

$$\sigma_{min}^2 = \frac{b^2}{12} + \sqrt{\frac{4}{105} \left(2D_0L + \frac{2S^2D_0}{L}\right) \left\{ \frac{S^2d^2}{(1-\alpha)^2D_0L} \right\}} \quad (23)$$

Notice that for pressure-driven backflows originating due to blockage of transverse EOF in an FFZE chamber, α varies between -1 and 1 based on our definition for this parameter. In our formulation, α assumes a negative value whenever the electrophoretic and electroosmotic analyte velocities oppose each other with the former exceeding the latter. In this situation, Eq. (22) suggests that $\bar{U}_{z, opt}$ can be over- and underestimated by factors between $1 - \infty$ and $1 - 2$ for positive and negative values of α , respectively, if the pressure-driven backflow in the system is ignored. Interestingly, the present analysis is also applicable to FFZE assays with a pressure-driven cross-flow that does not originate due to blockage of transverse EOF. In such a scenario, α can vary between $-\infty$ to ∞ causing both the over- and underestimation factors range from 1 to ∞ .

3.4 Electrodynamic dispersion in free-flow isoelectric focusing assays

Pressure-driven cross-flows can arise in free-flow isoelectric focusing (FFIEF) assays due to a non-uniform electroosmotic flow velocity along the channel width induced by the pH gradient in this direction. In addition, variations in the channel cross-section as well as unwanted differences in hydrostatic heads at the buffer/sample inlet ports can also lead to such pressure-gradients which besides altering the equilibrium position of the sample zones have a tendency to substantially broaden their widths deteriorating the separations. As a result, the analyte velocity in the x -direction (u_x) in FFIEF systems with a pressure-driven cross-flow can be assumed to comprise two additive contributions from the electrokinetic and pressure-driven cross-flows as given by $u_x = u_{EK} + u_c$ [9]. Moreover, the electrokinetic contribution (u_{EK}) can be assumed to originate both due to electrophoresis and electroosmosis of the analyte molecules in the x -direction with the gradient u_{EK}/x dominated by the electrophoretic component. Such a situation would be desirable in an FFIEF device as gradients in electroosmotic flow automatically produce unwanted pressure-driven cross-flows in the system leading to additional stream broadening. The transverse electrokinetic velocity under these conditions may be expressed as $u_{EK} = -k_{EP}x + U_{EOF}$ around the analyte's equilibrium position with U_{EOF} being the local electroosmotic flow component. An appropriate choice for the constant k_{EP} governing the electrophoretic component in an FFIEF chamber is $\mu E \omega / W$ where μ refers to absolute change in the electrophoretic mobility of the solute molecules across the width of the analysis chamber (W). The parameter ω here denotes the local dimensionless gradient in the analyte's electrophoretic mobility (μ_{EP}) with respect to the x -coordinate around its equilibrium position and assumes a value of 1 for a uniformly linear variation in μ_{EP} across the channel width. Notice that the minus sign associated with the electrophoretic cross-flow velocity in our analysis arises from the fact that the electric field in an FFIEF system is always applied in the direction of decreasing electrophoretic mobility for the solute molecules in order to drive them towards their equilibrium position. The gradient in the local pressure-driven cross-flow velocity u_c/x around the analyte's equilibrium position in this situation may be neglected and the quantity u_c expressed as $(3\bar{U}_c/2)(1 - 4y^2/d^2)$ with $\bar{U}_c$ being its spatially averaged value with respect to the y -coordinate (see Note 5). Also, recognizing the fact that the analyte stream in an FFIEF device flows along the z -direction under equilibrium conditions due to a net zero transverse velocity further allows one to drop all concentration gradients with respect to the z -coordinate in the system. Finally, the diffusion coefficient D may again be assumed to be uniform at all locations, i.e., $D = D_0$ due to a uniform temperature in the system leading to equations governing ϕ_p and m_p given by

$$pPe_{EP}\phi_p - p\phi_{p-1} - \frac{3}{2}pPe_c(1 - 4y^{*2})\phi_{p-1} = p(p-1)\phi_{p-2} + \frac{d^2\phi_p}{dy^{*2}} \quad (24)$$

$$pPe_{EP}m_p - pm_{p-1} - \frac{3}{2}pPe_c \int_{-1/2}^{1/2} (1 - 4y^{*2})\phi_{p-1} dy^* = p(p-1)m_{p-2} \quad (25)$$

The quantities $Pe_{EP} = \mu E \omega d^2 / W D_0$, $Pe_{EOF} = U_{EOF} d / D_0$, and $Pe_c = \bar{U}_c d / D_0$ in the above equations denote the Péclet numbers based on the electrophoretic, electroosmotic and pressure-driven cross-flow velocities, respectively. Notice that because the analyte velocity at the stream equilibrium position is zero in an FFIEF system, it is more appropriate to capture the effect of analyte migration in the x -direction on the separation performance using the Péclet numbers defined above rather than Pe_x as noted in Eq. (2). With these considerations in mind, it can now be shown that Eq. (24) reduces to $d^2 \phi_0 / dy^{*2} = 0$ for $p = 0$ yielding a solution $\phi_0 = m_0 = \delta$ upon application of the relevant boundary conditions. The corresponding equation for $p = 1$ may be written as

$$\frac{d^2 \phi_1}{dy^{*2}} = Pe_{EP} \phi_1 - Pe_{EOF} \delta - \frac{3}{2} Pe_c (1 - 4y^{*2}) \delta \quad (26)$$

yielding a solution

$$\phi_1 = \frac{6Pe_c \delta \cosh(\sqrt{Pe_{EP}} y^*)}{Pe_{EP}^{3/2} \sinh(\sqrt{Pe_{EP}}/2)} - \frac{6Pe_c \delta}{Pe_{EP}} y^{*2} + \frac{3}{2} \frac{Pe_c \delta}{Pe_{EP}} + \frac{Pe_{EOF} \delta}{Pe_{EP}} - 12 \frac{Pe_c \delta}{Pe_{EP}^2} \quad (27)$$

The normalized lateral equilibrium position for the analyte stream in this situation is given by

$$\frac{m_1}{m_0} = \frac{Pe_{EOF} + Pe_c}{Pe_{EP}} \quad (28)$$

which represents the x^* -position where the transverse analyte velocity averaged over the y^* -coordinate vanishes, i.e. $\int_{-1/2}^{1/2} u_x dy^* = 0$. Proceeding further, the governing differential equation for $p = 2$ can be expressed as

$$\frac{d^2 \phi_2}{dy^{*2}} = 2Pe_{EP} \phi_2 - 2Pe_{EOF} \phi_1 - 3Pe_c (1 - 4y^{*2}) \phi_1 - 2\delta \quad (29)$$

which upon integration from $y^* = -1/2$ to $y^* = 1/2$ and subsequent rearrangement yields

$$m_2 = \frac{Pe_{EOF}(Pe_c + Pe_{EOF})\delta}{Pe_{EP}^2} + \frac{\delta}{Pe_{EP}} + \frac{3}{2} Pe_c \int_{-1/2}^{1/2} (1 - 4y^{*2}) \phi_1 dy^* \quad (30)$$

Finally, the spatial variance of a sample stream (σ^2) in an FFIEF system with a pressure-driven cross-flow may be shown to be given by

$$\frac{\sigma^2}{d^2} = \frac{m_2}{m_0} - \frac{m_1^2}{m_0^2} = \frac{1}{Pe_{EP}} + \frac{Pe_c^2}{Pe_{EP}^2} \left[72 \frac{\coth(\sqrt{Pe_{EP}}/2)}{Pe_{EP}^{3/2}} - \frac{144}{Pe_{EP}^2} - \frac{12}{Pe_{EP}} + \frac{1}{5} \right] \quad (31)$$

under equilibrium conditions. In order to understand the expression for σ^2 presented above, it is important to realize that this variance arises from the interplay between three different factors, i.e. spatial gradient in the transverse electrophoretic velocity, molecular diffusion and transverse pressure-driven velocity. While the gradient in the transverse electrophoretic velocity (first factor) in an FFIEF device tends to focus the solute molecules at their equilibrium position, molecular diffusion (second factor) counters this effect producing a steady-state concentration distribution. These two factors lead to a spatial variance represented by the first term in Eq. (31), i.e. $d^2/Pe_{EP} = DW/(\mu\theta)$, which is identical to the expression derived by Svensson [10] given that the local gradient in the electrophoretic mobility of the solute particles with respect to the x-coordinate is $\mu\theta/W$ in our analysis. The normalized spatial variance of the analyte stream (σ^2/d^2) in this situation is governed by a single dimensionless parameter Pe_{EP} which represents the ratio of the characteristic electrophoretic to diffusive migration rate of the sample molecules in the system. Introduction of a pressure-driven cross-flow (third factor) under these conditions perturbs the noted steady state in two ways. Firstly, it shifts the lateral position where the analyte molecules experience a net zero transverse velocity modifying the value of m_1 in the device. Moreover, because such pressure-gradients also induce a variation in the streamline velocity with the y-coordinate, this equilibrium position changes across the depth of the assay chamber. The latter effect produces additional broadening of the analyte stream that is captured by the terms additive to $1/Pe_{EP}$ in Eq. (31). The noted equation also predicts that the Péclet number based on the electroosmotic cross-flow velocity in an FFIEF device does not influence the broadening of the analyte stream under equilibrium conditions. This result is a consequence of the uniformity in the electroosmotic flow profile across the channel depth which modifies the equilibrium positions of all solute molecules to an equal extent. The magnitude and direction of this cross-flow however, alters the equilibrium position of the solute particles and therefore enters the expression for m_1 in our analysis.

As may be seen from Eq. (31), the contribution to stream variance arising from the pressure-driven cross-flow velocity in an FFIEF device scales with the square of the Péclet number based on the parameter $\bar{U}_c$ but again diminishes with increasing values of Pe_{EP} . The noted trends arise due to the dispersal effect of fluid shear and the focusing effect of the gradient in electrophoretic velocity across the channel width, which are measured in terms of the parameters Pe_c and Pe_{EP} respectively, in our analysis. In Fig. 4(a), the different contributions to σ^2 as well as the total stream variance in an FFIEF system have been plotted as a function of these two relevant Péclet numbers. The figure shows several interesting trends both for small and large values of Pe_c and Pe_{EP} . In the limit of $Pe_{EP} \ll 1$, for example, the contribution to σ^2 from the pressure-driven cross-flow is seen to scale with Pe_{EP} in an identical way as that under ideal FFIEF conditions, i.e. $\sim 1/Pe_{EP}$. This occurs as the term

$$72 \frac{\coth(\sqrt{Pe_{EP}}/2)}{Pe_{EP}^{3/2}} - \frac{144}{Pe_{EP}^2} - \frac{12}{Pe_{EP}} + \frac{1}{5} \rightarrow \frac{Pe_{EP}}{210} \quad (32)$$

in this regime yielding an expression for the stream variance given by

$$\frac{\sigma^2}{d^2} \approx \frac{1}{Pe_{EP}} \left(1 + \frac{Pe_c^2}{210} \right) \quad (33)$$

As may be noted, Eq. (33) predicts the pressure-driven cross-flow to increase the spatial variance of the analyte stream by a factor $1 + Pe_c^2/210$, identical to that known for regular pressure-driven flow systems. In this limit of small electrophoretic migration rates, cross streamline diffusion interacts with the parabolic profile of the pressure-driven cross-flow in the same manner as was described by Taylor and Aris [4, 5]. Consequently, the curves depicting the ideal stream variance and the additional contribution from transverse pressure-gradients in Fig. 4(a) are seen to line up parallel to each other for $Pe_{EP} \ll 1$. The value of Pe_c dictates their relative magnitude in this regime with the pressure-driven cross-flow contribution exceeding the ideal component when $Pe_c > \sqrt{210}$. In the opposite limit of $Pe_{EP} \gg 1$, the term

$$72 \frac{\coth(\sqrt{Pe_{EP}}/2)}{Pe_{EP}^{3/2}} - \frac{144}{Pe_{EP}^2} - \frac{12}{Pe_{EP}} + \frac{1}{5} \rightarrow \frac{1}{5} \quad (34)$$

yielding an expression for the stream variance given by

$$\frac{\sigma^2}{d^2} \approx \frac{1}{Pe_{EP}} \left(1 + \frac{1}{5} \frac{Pe_c^2}{Pe_{EP}} \right) \quad (35)$$

Under these conditions, the pressure-driven cross-flow contribution decays with increasing values of Pe_{EP} much more rapidly than its $1/Pe_{EP}$ counterpart eventually having the overall stream variance approach its ideal limit. Physically this occurs, as the pressure-driven cross-flow now becomes negligible relative to the electrophoretic migration rate in the system causing the additional stream broadening induced by the transverse pressure-gradient to become vanishingly small. Fig. 4(a) also shows the noted trends lead to a cross-over of the ideal and pressure-driven cross-flow components to σ^2 for operating conditions with $Pe_c > \sqrt{210}$. The value of Pe_{EP} where this cross-over occurs, denoted as Pe_{EP}^* , has been plotted in Fig. 4(b) as a function of the parameter, Pe_c , in the system. The figure shows Pe_{EP}^*

to approach zero when $Pe_c \rightarrow \sqrt{210}$ as expected, and increase for larger values Pe_c . In the limit of $Pe_c \gg \sqrt{210}$, the quantity $Pe_{EP}^* \approx Pe_c^2/5$ as predicted by Eq. (35).

3.5 Stream dispersion in free-flow zone electrophoresis with Joule heating effects

The use of an electric field in free-flow zone electrophoresis (FFZE) automatically leads to Joule heating yielding a higher temperature at the center of the separation chamber relative to that around the channel walls. For small amounts of heat generated, this thermal effect introduces a variation in the equilibrium position of the analyte molecules due to the dependence of liquid viscosity and analyte diffusivity on temperature leading to a modification in the position of the analyte stream as well as the zone width [11]. The Joule heating of the background electrolyte under these conditions leads to a fully developed temperature (T) profile across the channel gap determined by the following expression based on Fourier's law

$$\frac{d}{dy} \left(k \frac{dT}{dy} \right) = -\lambda E^2 \quad \text{boundary conditions: } T = T_0 \quad \text{at } y = \pm \frac{d}{2} \quad (36)$$

where T_0 refers to the absolute temperature at the parallel plates and the quantities k and λ denote the ionic and thermal conductivities of the background electrolyte, respectively. It is important to point out that although the conductivity parameters k and λ in Eq. (36) are both functions of temperature, their dependence on T for aqueous solutions are significantly different from each other. For example, while the thermal conductivity of water is known to increase by less than 1% for small variations in T ($< 10^\circ\text{C}$) around room temperature (25°C), the corresponding change in λ can be as large as 25%. In this situation, the leading order effect of Joule heating may be captured by assuming k to be spatially constant (0.6 W/m/K for water at $T_0 = 25^\circ\text{C}$) [11] and the ionic conductivity to vary as $\lambda = \lambda_0 I_0 [1 + \alpha(T - T_0)]$ where $\lambda_0 = 0.14 \text{ m}^2\text{S/mol}$ (for aqueous KCl solutions), $\alpha = 0.025 \text{ /K}$ (for water) and I_0 equals the ion concentration in the background electrolyte in mol/m^3 [12].

Notice that the current analysis ignores any axial temperature gradients in the FFZE chamber due to an incompletely developed thermal profile, which for microchip based capillary zone electrophoretic devices, have been experimentally shown to exist only around the channel terminals extending about a few millimeters into the duct [13]. The results presented here are therefore strictly applicable to the middle section of the assay compartment with lengths about a centimeter or more, where the end effects from the channel terminals may be neglected. Fortunately, most microchip based FFZE chambers are several centimeters long in which case the overall stream dispersion in them is likely to be dominated by zone broadening mechanisms applicable to the central region of the chamber where the thermal profile is completely developed. In addition, a temperature gradient across the channel width (x -direction) of an FFZE chamber can arise either due to heat loss through its sidewalls or when the electrical and thermal properties of the flow buffer used is significantly different from that of the sample stream. Interestingly, the effect of heat loss through the channel sidewalls on sample transport may be neglected for large aspect ratio FFZE chambers (channel width to depth ratio $\gg 1$) especially when the sample stream is

conveyed far away from these edges. Again, this situation is often applicable to microchip based FFZE systems, and therefore has been considered in the present analysis. Moreover, the current work also ignores any significant differences in the electrical and thermal properties of the sample stream relative to that of the flow buffer unlike in isoelectric focusing systems for example. As a result, the thermal profile relevant to the stream dispersion process in FFZE assays is mostly dictated by energy transport across the narrower dimension in the separation chamber (y -direction) as has been considered in Eq. (36).

Proceeding further, Eq. (2) may now be normalized to the form

$$\frac{d^2\theta}{dy^{*2}} = -(1 + \alpha \Delta T \theta) \quad \text{boundary conditions: } \theta = 0 \quad \text{at } y^* = \pm \frac{1}{2} \quad (37)$$

after accounting for the variation in the ionic conductivity with temperature where $\theta = (T - T_0)/\Delta T$, $\Delta T = \lambda_0 I_0 E^2 d^2/k$ and $y^* = y/d$ (see Notes 6 and 7). It is well known that Eq. (37) admits a closed-form solution which for small values of ΔT , predicts θ to vary quadratically with y^* as noted below

$$\theta = \frac{1}{\alpha \Delta T} \left[\frac{\cos(\sqrt{\alpha \Delta T} y^*)}{\cos(\sqrt{\alpha \Delta T}/2)} - 1 \right] = \frac{1 - 4y^{*2}}{8\cos(\sqrt{\alpha \Delta T}/2)} + O(\Delta T^2) \quad (38)$$

Eq. (38) shows that Joule heating between two parallel plates leads to a parabolic thermal profile with the temperature differential between the channel center and the walls scaling with ΔT for small values of this parameter, i.e., $\Delta T < 25^\circ\text{C}$. Moreover, the noted temperature differential assumes values less than $0.14 \Delta T$, i.e., $< 3.5^\circ\text{C}$, under these conditions which are particularly relevant to microfluidic separation systems with channel depths in the range of $10 - 100 \mu\text{m}$. To highlight this point, the dependence of ΔT on the electric field (E) in an FFZE device has been plotted in Fig. 5(a) for different channel depths (d) and ion concentrations in the background electrolyte (I_0) commonly employed in microfluidic assays. The figure shows ΔT to assume values mostly under 25°C for d and I_0 in the range of $10 - 25 \mu\text{m}$ and $10 - 25 \text{ mM}$, respectively.

Having determined the temperature profile between the parallel plates, sample transport in this system may be analyzed by considering the analyte velocity along the x -direction (u_x) to comprise two additive contributions from electroosmosis and electrophoresis, i.e.,

$$u_x = \mu_{EP} E + \mu_{EOF} E. \text{ Interestingly however, while the non-uniformity in liquid temperature}$$

described by Eq. (38) produces a variation in the electrophoretic component across the channel gap, the electroosmotic counterpart remains spatially uniform provided the thickness of the Debye layer around the channel walls can be neglected relative to d . In this situation, the spatial variation in the quantity u_t may be expressed as

$$u_x = \mu_{EP}^0 E \eta_0 / \eta(y^*) + \mu_{EOF}^0 E \text{ where } \mu_{EP}^0 \text{ and } \mu_{EOF}^0 \text{ refer to the values of } \mu_{EP} \text{ and } \mu_{EOF} \text{ at the}$$

wall temperature T_0 , respectively. Now approximating the properties of the background electrolyte by those of water, the spatial variation in η may be captured using the correlation

$$\eta = A \exp\left(\frac{B}{T}\right) = \eta_0 \left(1 - \frac{B\Delta T\theta}{T_0^2}\right) = \eta_0 \left[1 - \frac{B\Delta T(1 - 4y^{*2})}{8T_0^2 \cos(\sqrt{\alpha\Delta T}/2)}\right] + O(\Delta T^2) \quad (39)$$

where $A = 2.76 \times 10^{-6}$ Pa s, $B = 1713$ K and $\eta_0 = A \exp(B/T_0)$.

The spatial variation in u_z may be determined by solving the Navier-Stokes equation while accounting for the temperature dependence of liquid viscosity yielding

$u_z = \bar{U}_z^0 \left[3(1 - 4y^{*2})/2 + 3\beta(1 - 4y^{*2})^2/4\right]$ to the leading order in T where $\bar{U}_z^0$ denotes the spatially averaged pressure-driven flow velocity at the wall temperature T_0 . To capture the spatial variation in D on the other hand, the Stokes-Einstein relationship for solute diffusivity, i.e., $D = k_B T / (6\pi\eta R)$, has been used in the present analysis where the symbols k_B and R correspond to the Boltzmann constant and the effective radius of the analyte molecule, respectively. In this situation, the equations governing ϕ_p and m_p may be written as

$$-pPe_{EP}f\phi_{p-1} - pPe_{EOF}\phi_{p-1} + Pe_z g \frac{\partial \phi_p}{\partial z^*} = p(p-1)h\phi_{p-2} + \frac{\partial}{\partial y^*} \left(h \frac{\partial \phi_p}{\partial y^*} \right) + h \frac{\partial^2 \phi_p}{\partial z^{*2}} \quad (40)$$

$$\begin{aligned} & -pPe_{EP} \int_{-1/2}^{1/2} f\phi_{p-1} dy^* - pPe_{EOF} m_{p-1} + Pe_z \int_{-1/2}^{1/2} \left(g \frac{\partial \phi_p}{\partial z^*} \right) dy^* \\ & = p(p-1) \int_{-1/2}^{1/2} h\phi_{p-2} dy^* + \int_{-1/2}^{1/2} \left(h \frac{\partial^2 \phi_p}{\partial z^{*2}} \right) dy^* \end{aligned} \quad (41)$$

where the functions $f = \eta_0/\eta = 1 + \beta(1 - 4y^{*2})$, $g = u_p/\bar{U}_z^0 = 3(1 - 4y^{*2})/2 + 3\beta(1 - 4y^{*2})^2/4$ and $h = D/D_0 = 1 + \gamma(1 - 4y^{*2})$ describe the spatial variation in the analyte electrophoretic velocity, liquid pressure-driven flow speed and analyte diffusivity, respectively, to the leading order in T . The various parameters relevant to these functions are defined as $\beta = B\Delta T / \{8T_0^2 \cos(\sqrt{\alpha\Delta T}/2)\}$, $\gamma = \Delta T(1 + B/T_0) / \{8T_0 \cos(\sqrt{\alpha\Delta T}/2)\}$, $\bar{U}_z^0 = \Delta P d^2 / (12\eta_0 L)$ and $D_0 = k_B T_0 / (6\pi\eta_0 R)$ with P being the pressure-drop applied across the axial length of the FFZE chamber (L) and D_0 referring to the analyte diffusivity at the wall temperature T_0 . The three Péclet numbers included in Eq. (40) here offer a measure of solutal advection via electrophoresis, electroosmosis and pressure-driven flow relative to their diffusive migration in the system and have been evaluated as $Pe_{EP} = \mu_{EP}^0 Ed/D_0$, $Pe_{EOF} = \mu_{EOF}^0 Ed/D_0$ and $Pe_z = \bar{U}_z^0 d/D_0$, respectively. For simplicity, the above expressions assume the radius of the analyte molecules (R) as well as the electrical charge carried by them to be unaffected by Joule heating which is appropriate for assessing sample transport in electrophoretic systems

with a small temperature differential across the channel gap. Notice that because the analyte velocity across the channel width in this analysis has an electrophoretic contribution that varies spatially and an electroosmotic one that does not, it is more appropriate to capture the effect of analyte migration in the x -direction on the separation performance using 2 separate parameters Pe_{EP} and Pe_{EOF} as defined above rather than a single one, e.g., Pe_x as noted in Eq. (2). As may be noted, Eq. (40) suggests that the leading order effects of Joule heating on the separation performance of FFZE assays are captured by the dimensionless parameters β and/or γ which are both proportional to the quantity T . In this situation, it is important to have a sense of the magnitude of these quantities which have been included in Fig. 5(b). The figure shows that for aqueous electrolytes (with $B = 1713$ K) and a wall temperature of $T_0 = 298$ K, the parameter γ always exceeds β by about 17% ($= T_0 \times 100/B$) with both quantities assuming values less than 0.1 under operating conditions commonly applicable to microfluidic FFZE assays.

The integral boundary condition in Eq. (3) implies that ϕ_0 , and hence m_0 , cannot be functions of z^* . Upon incorporating this result into the differential equation governing ϕ_0 , it can then be shown $d(h d\phi_0/dy^*)/dy^* = 0$ yielding the solutions $\phi_0 = m_0 = \delta$. The corresponding equation for ϕ_1 in this situation becomes

$$Pe_z g \frac{\partial \phi_1}{\partial z^*} - h \frac{\partial^2 \phi_1}{\partial z^{*2}} - \frac{\partial}{\partial y^*} \left(h \frac{\partial \phi_1}{\partial y^*} \right) = Pe_{EP} f \delta + Pe_{EOF} \delta \quad (42)$$

which is solved by assuming ϕ_1 to vary linearly with z^* , i.e., $\phi_1 = \psi_{11} z^* + \psi_{10}$. Upon substituting this functional form of ϕ_1 in Eq. (42) and later matching the coefficients of z^* , it is possible to show that $d(h d\psi_{11}/dy^*)/dy^* = 0$ which admits the following solution to the leading order in T (see Note 8)

$$\psi_{11} = \frac{(Pe_{EP} + Pe_{EOF})\delta}{Pe_z} + \frac{2(2Pe_{EP} - 3Pe_{EOF})\beta\delta}{15Pe_z} \quad (43)$$

It must be noted that the parameters β and γ both scale with the quantity T in the present analysis, and are therefore involved in terms that capture the leading order effect of Joule heating on sample transport. Proceeding further, the terms independent of z^* in Eq. (42) are equated to obtain $d(h d\psi_{10}/dy^*)/dy^* = Pe_z g \psi_{11} - Pe_{EP} f \delta - Pe_{EOF} \delta$ which is solved to the leading order in T yielding

$$\begin{aligned} \psi_{10} = & (Pe_{EP} + Pe_{EOF})\delta \left[\frac{(120y^{*2} - 240y^{*4} - 7)}{480} \right. \\ & \left. + \left(\frac{\beta}{5600} - \frac{\gamma}{1680} \right) (420y^{*2} - 1680y^{*4} + 2240y^{*6} - 19) \right] \end{aligned} \quad (44)$$

The normalized lateral equilibrium position of the analyte stream in this situation can be evaluated as

$$\frac{m_1}{m_0} = \left[\frac{(Pe_{EP} + Pe_{EOF})}{Pe_z} + \frac{2\beta(2Pe_{EP} - 3Pe_{EOF})}{15Pe_z} \right] z^* \quad (45)$$

which shows Joule heating to shift this quantity by an additional term proportional to β . Moreover, Eq. (45) predicts that the noted term can assume both positive and negative values depending on the amount of electroosmosis relative to electrophoresis in the system, allowing the analyte stream to drift either way of the equilibrium position realized in the absence of Joule heating. In the limit of diminishing electrophoresis ($Pe_{EP}/Pe_{EOF} = \mu_{EP}^0/\mu_{EOF}^0 \rightarrow 0$) for example, the analytic theory predicts the stream position to drift by an amount $-2\beta Pe_{EOF}/5$ implying a smaller deflection of the analyte stream by the electric field in the presence of Joule heating. This occurs as the lateral migration of the solute molecules under these conditions is completely dominated by electroosmosis which is unaffected by the temperature rise at the center of the channel. Nevertheless, for a given pressure-drop (P) across the channel length, the lower liquid viscosity in the device increases the sample velocity along the z -coordinate by a factor of $1 + 2\beta/5$ to the leading order reducing the residence time of the analyte stream by a factor $1 - 2\beta/5$ (see Fig. 6(a)). In the opposite scenario when the magnitude of μ_{EOF}^0/μ_{EP}^0 is small, i.e., $\mu_{EOF}^0/\mu_{EP}^0 \rightarrow 0$, the lateral position of the analyte molecules is completely determined by their electrophoretic migration along the x -coordinate which speeds-up by a factor of $1 + 2\beta/3$ to the leading order due to the reduced viscosity in the system. In this limit, the combined effects of the increased pressure-driven and electrophoretic flow speeds leads to a change in the quantity m_1/m_0 by a factor $1 + 4\beta/15$ as shown in Fig. 6(b). It must be noted that while the solid curves in Fig. 6(a)/(b) correspond to the predictions of the analytic theory, the dashed ones represent simulation results obtained by numerically solving Eq. (42) without linearizing the expressions for the liquid viscosity, analyte diffusivity and the temperature profile across the channel gap with respect to T . As expected, the deviation between the two curves is seen to grow for larger values of T with the analytic theory underpredicting the numerical simulations for the case of no electrophoresis. Nevertheless, this mismatch is still below 0.2% for $\beta = 0.1$ and the operating conditions considered in Fig. 6(a). Interestingly, the analytic theory overpredicts the simulation results in the opposite scenario of zero electroosmosis but the deviation between the two outcomes is even smaller for the conditions considered in Fig. 6(b).

Now for estimating the second moment of C^* , Eq. (40) is again referred back to arrive at the differential equation governing ϕ_2

$$Pe_z g \frac{\partial \phi_2}{\partial z^*} - h \frac{\partial^2 \phi_2}{\partial z^{*2}} - \frac{\partial}{\partial y^*} \left(h \frac{\partial \phi_2}{\partial y^*} \right) = 2Pe_{EP} f \phi_1 + 2Pe_{EOF} \phi_1 + 2h\delta \quad (46)$$

which is solved by assuming the functional form $\phi_2 = \psi_{22}z^{*2} + \psi_{21}z^* + \psi_{20}$. Upon substituting this quadratic polynomial in z^* into Equation (46) and later matching the coefficients for z^{*2} , it can be shown that $d(h d\psi_{22}/dy^*)/dy^* = 0$ which admits the following solution to the leading order in T

$$\psi_{22} = \frac{(Pe_{EP} + Pe_{EOF})^2 \delta}{Pe_z^2} + \frac{4(Pe_{EP} + Pe_{EOF})(2Pe_{EP} - 3Pe_{EOF})\beta\delta}{15Pe_z^2} \quad (47)$$

Later matching the coefficients for z^* in Equation (46) yields

$d(hd\psi_{21}/dy^*)/dy^* = 2Pe_z g\psi_{22} - 2Pe_{EP} f\psi_{11} - 2Pe_{EOF} \psi_{11}$ which is solved to the leading order in T to obtain

$$\begin{aligned} \psi_{21} = & \frac{(Pe_{EP} + Pe_{EOF})^2 \delta (280y^{*2} - 560y^{*4} - 11)}{560Pe_z} + \frac{2\delta}{Pe_z} \left[1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} \right] \quad (48) \\ & + \frac{(Pe_{EP} + Pe_{EOF})\delta\beta}{25200Pe_z} \left[Pe_{EP}(20160y^{*6} - 21840y^{*4} + 7140y^{*2} - 367) \right. \\ & + Pe_{EOF}(20160y^{*6} - 5040y^{*4} - 1260y^{*2} + 123) \left. \right] \\ & + \frac{(Pe_{EP} + Pe_{EOF})^2 \delta \gamma (5040y^{*4} - 6720y^{*6} - 1260y^{*2} + 41)}{2520Pe_z} - \frac{4\delta\beta}{5Pe_z} \\ & + \frac{4\delta\gamma}{3Pe_z} \left[1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} \right] + \frac{4(Pe_{EP} + Pe_{EOF})\delta\beta(Pe_{EP} - 9Pe_{EOF})}{15Pe_z^3} \end{aligned}$$

Similarly, it is possible to determine the spatial dependence of ψ_{20} on y^* by equating the terms independent of z^* after substituting the functional form of ψ_2 in Eq. (46). However, it is important to realize that the second moment of C^* , i.e., m_2 , only depends on the spatially averaged value of ψ_{20} , i.e., $\int_{-1/2}^{1/2} \psi_{20} dy^*$, which interestingly is simply equal to $\delta^2/12$ as the analyte molecules are assumed to be uniformly distributed over a normalized width δ at the channel inlet ($z^* = 0$). Combining these results with the integrals of Eqs. (47) and (48) over the channel gap, the second moment of C^* in any $x - y$ plane may be estimated as

$$\begin{aligned}
m_2 = & \frac{(Pe_{EP} + Pe_{EOF})^2 \delta}{Pe_z^2} \left[1 + \frac{4(2Pe_{EP} - 3Pe_{EOF})\beta}{15(Pe_{EP} + Pe_{EOF})} \right] z^{*2} \quad (49) \\
& + \left[\frac{2\delta}{Pe_z} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \right. \\
& + \frac{4\delta\gamma}{3Pe_z} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} - \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \\
& \left. + \frac{4\delta\beta(Pe_{EP} + Pe_{EOF})(Pe_{EP} - 9Pe_{EOF})}{15Pe_z^3} - \frac{4\delta\beta}{5Pe_z} \right] z^* + \frac{\delta^3}{12}
\end{aligned}$$

The normalized spatial variance of the sample stream (σ^2/d^2) evaluated as $m_2/m_0 - m_1^2/m_0^2$ in this situation turns out to be

$$\begin{aligned}
\sigma^{*2} = \frac{\sigma^2}{d^2} = & \left[\frac{2}{Pe_z} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \right. \quad (50) \\
& + \frac{4\beta}{5Pe_z} \left\{ \frac{(Pe_{EP} + Pe_{EOF})(Pe_{EP} - 9Pe_{EOF})}{3Pe_z^2} - 1 \right\} \\
& \left. + \frac{4\gamma}{3Pe_z} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} - \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \right] z^* + \frac{\delta^2}{12}
\end{aligned}$$

which shows this quantity to increase linearly with the axial position (z -coordinate) in the separation chamber as is the case in the absence of Joule heating. The factor multiplying z^* in this equation is a measure of the effective dispersion coefficient for the analyte zone with the term $\delta^2/12$ representing the contribution from the injection width. Eq. (50) also shows that the leading order effect of Joule heating on the stream variance in FFZE assays is captured by two terms proportional to β and γ which both scale with T . While the first of these two terms quantitates the effect of a modified electrophoretic and pressure-driven flow velocity due to a lower liquid viscosity around the channel center, the second one captures the influence of an increased analyte diffusivity in the system. Fig. 7 shows that Joule heating tends to reduce the overall stream broadening in FFZE assays in the limit when this quantity is dominated by the hydrodynamic dispersion component, i.e.,

$Pe_z, Pe_{EP} + Pe_{EOF} \gg \sqrt{210}$. This result may be best analyzed in terms of the Taylor-Aris dispersion coefficient (K) in the system which relates to the stream variance as $\sigma^2 = 2Kt + b^2/12$ where $t = z^*d(1 - 2\beta/5)/\bar{U}_p^0$ denotes the residence time of the analyte stream in the FFZE chamber. Upon incorporating this information in Eq. (50) and making the relevant rearrangement, it is now possible to express

$$\begin{aligned}
 K = D_0 & \left[1 + \left(\frac{Pe_{EP} + Pe_{EOF}}{Pe_z} \right)^2 + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right. \\
 & + \frac{2\beta}{5} \left[\frac{2(Pe_{EP} + Pe_{EOF})(2Pe_{EP} - 3Pe_{EOF})}{3Pe_z^2} + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right] \\
 & \left. + \frac{2\gamma}{3} \left[1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_z^2} - \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right] \right] \quad (51)
 \end{aligned}$$

Equation (51) shows that the modified electrophoretic and pressure-driven flow profile in the presence of Joule heating alters both the orientation factor as well as the hydrodynamic dispersion contributions in the system. In fact, the term

$4\beta D_0(Pe_{EP} + Pe_{EOF})(2Pe_{EP} - 3Pe_{EOF})/(15Pe_z^2)$ exactly accounts for the change in the orientation factor to the leading order in T based on the drift in the lateral equilibrium stream position predicted by Eq. (45). The other term scaling with β in Eq. (51), i.e., $\beta D_0(Pe_{EP} + Pe_{EOF})^2/525$, captures the effect of Joule heating on the hydrodynamic dispersion component and always assumes a positive value due to an increased shear in the system. The terms proportional to the parameter γ in Eq. (51) quantitate the influence of an increased analyte diffusivity under the operating conditions. In particular, this expression shows that faster diffusion along the x -coordinate broadens the sample zone by an additional term given by $2\gamma D_0 \left[1 + (Pe_{EP} + Pe_{EOF})^2/Pe_z^2 \right]/3$. Whereas the increased diffusivity along the y -coordinate forces a reduction in the hydrodynamic dispersion component by an amount equal to $\gamma D_0(Pe_{EP} + Pe_{EOF})^2/315$. Such a reduction occurs as analyte molecules are now able to equilibrate at a faster rate across the different flow streamlines compared to the situation when Joule heating is absent. Overall however, the subtractive hydrodynamic dispersion component arising from faster analyte diffusion offsets the corresponding additive contribution resulting from larger fluid shear as γ always exceeds β based on their definitions (see Fig. 5(b)). Fig. 7 also shows that Joule heating can be expected to reduce stream broadening by about 20% in the absence of electrophoresis under conditions when $Pe_z = 1000$ and $Pe_{EOF} = 2000$ with the analytic theory underpredicting the numerical simulation results by around 5%. However when electroosmosis is eliminated from the system and $Pe_{EP} = 2000$, the reduction in zone dispersion due to Joule heating is as much as 36% for the same value of Pe_z with the analytic theory now overpredicting the simulation results by almost 20%. It must be pointed out that the Péclet numbers noted above may be realized in a 25 μm deep FFZE channel for a choice of $\bar{U}_p^0 = 1 \text{ cm/s}$, $E = 8 \text{ kV/cm}$ and μ_{EP}^0 or $\mu_{EOF}^0 = 3 \times 10^{-4} \text{ cm}^2/\text{Vs}$.

4. Concluding remarks

While the mathematical formulation described in this chapter has been applied to evaluating the mean position and spatial variance of a sample stream in free-flow electrophoretic assays, it can also be extended to calculating the higher order moments of the analyte concentration profile, e.g., skewness, kurtosis, etc. However, the present analysis is only

valid for assays in which the transit time for the analyte molecules in the separation chamber is much larger compared to their characteristic diffusion time across the gap between the parallel plates, i.e., $L/\bar{U}_z \gg d^2/D_0$. Moreover, our mathematical model ignores the effect of the channel sidewalls on the pressure-driven flow profile, and hence on the hydrodynamic dispersion component of the sample stream. Furthermore, it does not account for any additional band broadening introduced by the abrupt changes in the channel cross-section at the entrance and exit of the separation chamber. Fortunately, these effects tend to be insignificant in most free-flow electrophoretic systems rendering the current mathematical model highly useful in assessing the performance of this fractionation method.

5. Notes

1. The parallel-plate geometry considered here ignores the effect of channel sidewalls on fluid and analyte transport. In this situation, the present analysis is only valid for large aspect ratio separation chambers, i.e., $d/W \ll 1$, and in regions significantly away from the sidewalls.
2. The analyte concentration and liquid flow profiles have been assumed to be decoupled in our mathematical model. This decoupling of the mass and momentum conservation equations is strictly valid for dilute samples for which the solute concentration profile does not influence any of the material properties in the system.
3. The choice of the condition $m_1|_{z^*=0} = 0$ is not essential but simplifies the mathematical expressions in our analysis by introducing the sample stream at the origin of the x -coordinate.
4. The condition $m_2|_{z^*=0} = \delta^3/12$ assumes that analyte concentration (C) is uniform across the x - and y -coordinates at the channel entrance $z = 0$.
5. For a channel of uniform cross-section, if the pressure-driven cross-flow (u_c) originates due to a variation in EOF along the x -coordinate induced by the pH gradient in the system, the quantity u_c/x may be ignored provided $U_{EOF}/x \ll u_{EK}/x$.
6. The thermal profile in a straight microchamber may be calculated independently of the velocity field provided the temperature gradient along the x - and z -coordinates and the advective velocity along the y -coordinate may be neglected.
7. The transverse advective velocity component along the y -coordinate may be neglected for small Reynolds number flows in the absence of any natural convection in the channel (small Grashof number).
8. The higher order terms in T may be estimated following a similar method by retaining the higher order terms in the expressions for η and D in our analysis.

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Figure 1. Schematic of the free-flow electrophoretic separation process between two parallel-plates as described in this chapter. Reproduced with permission from reference [3].

Figure 2. Contributions to stream variance by the various factors in a FFZE system **(a)** for a constant lateral electric field ($E = 500 \text{ V/cm}$). **(b)** for a constant lateral distance migrated by the analyte molecules before exiting the separation chamber ($S = 5 \text{ mm}$). The values for the other operating parameters in both these figures were chosen to be $d = 20 \text{ }\mu\text{m}$, $D = 8 \times 10^{-6} \text{ cm}^2/\text{s}$, $\mu = 5 \times 10^{-4} \text{ cm}^2/\text{Vs}$, $L = 2.5 \text{ cm}$ and $b = 100 \text{ }\mu\text{m}$. Reproduced with permission from reference [3].

Figure 3.

(a) Fitting of the experimentally measured stream variance reported by Fonslow and Bowser [6] to an equation of the form $\sigma^2 = A + B/\bar{U}_z + C\bar{U}_z$. (b) Estimated values of A , B and C

from the regression analysis presented in (a) and fitting of the parameter C to an equation of the form $C = kS^2$. Reproduced with permission from reference [3].

Figure 4.

(a) Dependence of the equilibrium stream variance on the quantity Pe_{EP} as predicted by Eq. (31) in the presence of a steady pressure-driven cross-flow in an FFIEF system. “Ideal conditions” here correspond to the situation when $Pe_c = 0$. (b) Variation in the value of Pe_{EP}^* as function of Pe_c in an FFIEF assay with a steady pressure-driven cross-flow. The quantity Pe_{EP}^* here denotes the value of Pe_{EP} at which the additional spatial variance from the pressure-driven cross-flow equals that expected under ideal conditions, i.e. d^2/Pe_{EP} . Reproduced with permission from reference [9].

Figure 5.

(a) Estimated characteristic temperature rise as a function of the applied electric field in an FFZE device. (b) Estimated values of the dimensionless parameters (β and γ) that determine the effect of Joule on analyte transport in an FFZE system. Reproduced with permission from reference [11].

Figure 6. Effect of electric field on the drift in the lateral position of the analyte stream due to Joule heating in the absence of (a) electrophoresis (b) electroosmosis. Reproduced with permission from reference [11].

Figure 7.

Effect of electric field on the spatial variance of the analyte stream in the absence of (a) electrophoresis (b) electroosmosis. In the above figures, the quantity $\sigma^* = \sigma/d$. Reproduced with permission from reference [11].