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Broadening of Analyte Streams due to a Transverse Pressure Gradient in Free-Flow Isoelectric Focusing

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Abstract

Pressure-driven cross-flows can arise in free-flow isoelectric focusing systems (FFIEF) 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. In this situation, a thorough assessment of stream broadening due to transverse pressure-gradients in FFIEF devices is necessary in order to establish accurate design rules for the assay. The present article describes a mathematical framework to estimate the noted zone dispersion in FFIEF separations based on the method-of-moments approach under laminar flow conditions. A closed-form expression has been derived for the spatial variance of the analyte streams at their equilibrium positions as a function of the various operating parameters governing the assay performance. This expression predicts the normalized stream variance under the chosen conditions to be determined by two dimensionless Péclet numbers evaluated based on the transverse pressure-driven and electrophoretic solute velocities in the separation chamber, respectively. Moreover, the analysis shows that while the stream width can be expected to increase with an increase in the value of the first Péclet number, the opposite trend will be followed with respect to the latter. The noted results have been validated using Monte Carlo simulations that also establish a time/length scale over which the predicted equilibrium stream width is attained in the system.

Keywords

band broadening; free-flow isoelectric focusing; hydrodynamic dispersion; method-of-moments; pressure-driven cross-flow

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1. Introduction

Continuous flow separation of sample components is advantageous in several applications involving the analysis of complex chemical and biological mixtures particularly as a upstream analyte enrichment step [1-3]. These include larger throughputs, real-time readout of the separation efficiency enabling on-line feedback for process optimization, and continuous as well as independent collection of the sample fractions [4,5]. Of these, the third advantage is particularly important as it allows upstream or downstream integration of such separation methods to other analysis procedures with relative ease. Continuous flow separation techniques rely on a steady introduction of the sample stream co-currently flowing with an appropriate buffer solution which is then subjected to a force field acting at an angle, often perpendicular, to its flow direction. In this situation, the various components of the sample mixture experiencing different lateral deflections take different paths through the analysis chamber resulting in their spatial separation at the channel outlet. Over the years, a variety of continuous separation methods have been developed based on this approach relying on lateral deflections induced by electric [6-9], magnetic [10-12], hydrodynamic [13], acoustic [14,15], and optical [16,17] forces. Among these, the electrically-driven techniques are arguably the most widely used ones in academic research and industrial applications due to the simplicity with which electric fields can be applied at different length scales.

Free-flow isoelectric focusing (FFIEF) is an important electrically-driven continuous flow separation method that exploits the differences in the isoelectric points of the sample components to isolate them [18]. In this technique, a pH gradient is introduced perpendicular to the sample flow in conjunction with an electric field applied along this gradient. The different analyte streams under these conditions are laterally deflected to a position where the local pH equals their isoelectric point leading to the desired spatial separation. FFIEF is particularly notable for producing relatively narrow analyte zones due to the electrophoretic focusing of the sample molecules around their equilibrium position, i.e. the region in which the local pH equals the analyte's isoelectric point [19,20]. As a result, this separation method has been successfully applied to analyzing a variety of complex biological mixtures including those of fluorescent IEF markers [21], proteins [8,22,23], oligonucleotides [24], mitochondria [25], and bacteria [26]. Despite its remarkable separation and focusing capabilities, several factors tend to limit the performance of the FFIEF assay. In particular, practical challenges to realizing stable pH gradients, preventing bubble-forming electrolysis products from entering the analysis chamber, as well as mitigating Joule heating effects often diminish the figures-of-merit for this analysis technique.

These non-idealities in an FFIEF system perhaps deteriorate the resolving power of the assay to the greatest extent by increasing the zone dispersion of the analyte streams [27,28]. Such dispersion under ideal conditions was mathematically analyzed for the first time by Svensson [29] who considered a linear variation in the electrophoretic mobility (μ) of the sample molecules around their equilibrium position. It was shown that for a steady lateral electric field (E), the equilibrium stream variance attained a value of $D/(pE)$ where D is the molecular diffusivity of the analyte species and $p = -d\mu/dx$ is the local gradient in its electrophoretic mobility along the channel width. The noted expression however, does not

account for any non-idealities in the device including a transverse pressure-driven flow across the width of the separation chamber. Remarkably, such pressure-gradients may not be completely prevented in an FFIEF system due to the changing pH across the width of the assay compartment which automatically produces a variation in the zeta potential at its top and bottom walls along this direction [30]. Consequently, a transverse electroosmotic flow is generated in the device whose magnitude changes along the pH gradient introducing a transverse pressure-driven flow in the system [31,32]. In addition, similar pressure-driven cross-flows may also originate in FFIEF chambers due to variations in the channel cross-section as well as unwanted differences in hydrostatic heads at the buffer/sample inlet ports. These cross-flows then modify the equilibrium position of the analyte stream besides broadening them to a significant extent [33,34] beyond that predicted by Svensson's model. In the current article, a mathematical analysis of the zone dispersion problem in FFIEF systems with a steady pressure-gradient in the transverse direction is presented to assess their separation performance. A method-of-moments approach [35,36] is taken to solve the relevant material balance equations yielding a closed-form expression for the spatial variance of the analyte stream under equilibrium conditions. The predictions of this mathematical expression are further validated using Monte Carlo simulations which also establish a time/length scale over which the noted mathematical expressions for stream width are valid.

2. Mathematical Formulation

To evaluate the spatial variance of a sample zone in an FFIEF system, the flow of an analyte stream between two parallel plates separated by a distance d has been considered (see Figure 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, the locations of the parallel plates have been assumed to be $y = \pm d/2$ yielding an axial pressure-driven velocity profile $u_a = (3U_a/2)(1 - 4y^2/d^2)$ with U_a being the spatially averaged value of u_a . The velocity field for the sample molecules in the x -direction (u_t) is assumed to comprise two additive contributions from the electrokinetic and pressure-driven cross-flows as given by $u_t = u_{EK} + u^P$. Moreover, the electrokinetic contribution (u_{EK}) is assumed to originate due to both electrophoresis and electroosmosis of the analyte molecules in the x -direction with the gradient $\partial u_{EK}/\partial x$ 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 being the local electroosmotic flow component. An appropriate choice for the constant K_{EP} governing the electrophoretic component in an FFIEF chamber is $\mu E \theta / W$ where μ refers to absolute change in the electrophoretic mobility of the solute molecules across the width of the analysis chamber (W). The parameter θ 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 [29]. The gradient in the local pressure-driven cross-flow velocity $\partial u_p / \partial x$ around the analyte's equilibrium position in this situation may be neglected and the quantity u_p expressed as $(3U_p/2)(1 - 4y^2/d^2)$ with U_p being its spatially averaged value with respect to the y -coordinate. 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. These considerations lead to an advection-diffusion equation governing the concentration of the sample species (C) in our assay chamber given by

$$-\frac{\Delta\mu E\theta}{W}\frac{\partial(xC)}{\partial x} + U_{EOF}\frac{\partial C}{\partial x} + \frac{3}{2}U_p\left(1 - 4\frac{y^2}{d^2}\right)\frac{\partial C}{\partial x} = D\left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2}\right) \quad (1)$$

Now upon normalizing all length scales with respect to d , i.e. $x^*, y^* = x/d, y/d$ and the sample concentration by its inlet value (C_0), i.e. $C^* = C/C_0$, Equation (1) may be reduced to the dimensionless form

$$-Pe_{EP}\frac{\partial(x^*C^*)}{\partial x^*} + Pe_{EOF}\frac{\partial C^*}{\partial x^*} + \frac{3}{2}Pe_p\left(1 - 4y^{*2}\right)\frac{\partial C^*}{\partial x^*} = \frac{\partial^2 C^*}{\partial x^{*2}} + \frac{\partial^2 C^*}{\partial y^{*2}} \quad (2)$$

The quantities $Pe_{EP} = \mu E \theta d^2 / WD$, $Pe_{EOF} = U_{EOF}d/D$, and $Pe_p = U_p d/D$ here denote the Péclet numbers based on the electrophoretic, electroosmotic and pressure-driven cross-flow velocities, respectively, yielding measures of advective transport based on these mechanisms relative to diffusive migration under the operating conditions. The boundary conditions relevant to Equation (2) can be written as $\partial C^* / \partial y^*$ at $y^* = \pm 1/2$ and C^* , $\partial C^* / \partial x^*$ as $x^* \rightarrow \pm\infty$ to arrive at a solution for the dimensionless concentration profile. In addition to these constraints, the amount of analyte flowing per unit time through any y -plane is set to a constant M in our analysis which can be equated to the integral

$\left(3C_0U_p d^2/2\right) \int_{-1/2}^{1/2} \int_{-\infty}^{\infty} \left(1 - 4y^2\right) C^* dx^* dy^*$ to satisfy the material balance in the system. Now upon multiplying Equation (2) with x^{*n} followed by integrating it along the x^* -coordinate from $-\infty$ to ∞ , it is possible to show that [35]

$$nPe_{EP}\phi_n - nPe_{EOF}\phi_{n-1} - \frac{3}{2}nPe_P(1 - 4y^{*2})\phi_{n-1} = n(n-1)\phi_{n-2} + \frac{d^2\phi_n}{dy^{*2}}$$

(3)

Boundary conditions: $\left. \frac{d\phi_n}{dy^*} \right|_{y^*=\pm 1/2} = 0$;

$$\int_{-1/2}^{1/2} (1 - 4y^{*2})\phi_0 dy^* = \frac{2M}{3C_0U_a d^2} = \frac{2\delta}{3} \text{ where } \phi_n = \int_{-\infty}^{\infty} x^{*n} C^* dx^*.$$

Further integrating Equation (3) along the y^* -coordinate over the region between the parallel plates and defining $m_n = \int_{-1/2}^{1/2} \phi_n dy^*$ one can obtain

$$nPe_{EP}m_n - nPe_{EOF}m_{n-1} - \frac{3}{2}nPe_P \int_{-1/2}^{1/2} (1 - 4y^{*2})\phi_{n-1} dy^* = n(n-1)m_{n-2}$$

(4)

Notice that the quantity m_n in this formulation represents the n^{th} moment of C^* in any x - y plane with m_1/m_0 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.

In order to validate the zone width for the analyte stream predicted by equations 3 and 4, Monte Carlo simulations were also carried out in the present study. In these simulations, the migration of 10^6 point-like particles was tracked between two parallel plates based on the deterministic velocity profile described by $\vec{u} = u_t \hat{e}_x + u_a \hat{e}_z$ coupled to a random walk diffusion model where $\hat{e}_x$ and $\hat{e}_z$ denote the unit vectors along the x and z -coordinates, respectively. Molecules at each time-step of size Δt were randomly displaced along the x , y and z -coordinates by an amount governed by a Gaussian distribution with a mean value equal to the local velocity along the corresponding coordinate times Δt and a standard deviation $\lambda = \sqrt{2D\Delta t}$. Furthermore, the molecules were appropriately reflected off the channel walls to capture the effect of the boundary conditions on stream dispersion. The gap width between the parallel plates in the Monte Carlo simulation was chosen to be one unit and with the domain extending 60 units in the axial pressure-driven flow direction ($0 \leq z^* \leq 60$). This FFIEF chamber was assumed to be unbounded along the x -coordinate to eliminate the effect of the channel sidewalls on the stream dispersion phenomenon. Because the noted simulations were primarily designed to capture the hydrodynamic dispersion contribution, the spatial variance of the injected stream was chosen to be at least 10^4 times smaller than its steady state value at the outlet of the FFIEF chamber. It was also ensured that the stream width at the channel outlet reached its equilibrium value by choosing the

length of the FFIEF chamber to be at least 8 times larger than the product of the axial stream velocity (U_a) and the characteristic diffusion ($d^2/4D$) as well as the equilibration ($W/\mu E\theta$) time scales in the system, i.e. $L \gg U_a d^2/4D, U_a W/\mu E\theta$. Finally, our simulations were initiated by assuming a Gaussian distribution of the analyte molecules around the equilibrium position of the sample stream (set to $x^* = 0$) with a spatial variance equal to that of the injected stream, and therefore, negligibly small compared to its steady state value. A constant analyte flow was maintained in the system by re-introducing all molecules exiting the FFIEF chamber at $Z^* = 60$ back into the compartment through its inlet with their starting x and y coordinates determined by the distribution chosen for the initial condition. The starting Z coordinate (Z_0^*) for these molecules was set equal to the distance it had migrated beyond the outlet, i.e. $Z_0^* = Z^* - L$ in the previous time step. The steady state spatial variance was estimated by running the simulations over a time period equal to 5 times the larger of the advection, diffusion and equilibration time scales in the system, i.e. $L/U_a, d^2/4D$, and $W/\mu E\theta$, respectively. All simulations included in this study were performed using codes written in MATLAB. The Monte Carlo code used for our simulations has been included as Supporting Information.

3. Results and Discussion

For $n = 0$, it can be shown that Equation (3) reduces to $d^2\phi_0/dy^{*2} = 0$ yielding a solution $\phi_0 = m_0 = \delta$ upon application of the relevant boundary conditions. The corresponding equation for $n = 1$ may be written as

$$\frac{d^2\phi_1}{dy^{*2}} = Pe_{EP}\phi_1 - Pe_{EOF}\delta - \frac{3}{2}Pe_P(1 - 4y^{*2})\delta \quad (5)$$

yielding a solution

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

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

$$\frac{m_1}{m_0} = \frac{Pe_{EOF} + Pe_P}{Pe_{EP}} \quad (7)$$

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

$$\frac{d^2\phi_2}{dy^{*2}} = 2Pe_{EP}\phi_2 - 2Pe_{EOF}\phi_1 - 3Pe_P(1 - 4y^{*2})\phi_1 - 2\delta \quad (8)$$

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

$$m_2 = \frac{Pe_{EOF}(Pe_P + Pe_{EOF})\delta}{Pe_{EP}^2} + \frac{\delta}{Pe_{EP}} + \frac{3}{2}Pe_P \int_{-1/2}^{1/2} (1 - 4y^{*2})\phi_1 dy^* \quad (9)$$

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

$$\frac{\sigma^2}{d^2} = \frac{m_2}{m_0} - \frac{m_1^2}{m_0^2} = \frac{1}{Pe_{EP}} + \frac{Pe_P^2}{Pe_{EP}^2} \left[72 \frac{\coth(\sqrt{Pe_{EP}}/2)}{Pe_{EP}^{3/2}} - \frac{144}{Pe_{EP}^2} - \frac{12}{Pe_{EP}} + \frac{1}{5} \right] \quad (10)$$

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 Equation (10), i.e. $d^2/Pe_{EP} = DW/(\mu E\theta)$, which is identical to the expression derived by Svensson [29] 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 [36]. The latter effect produces additional broadening of the analyte stream that is captured by the terms additive to $1/Pe_{EP}$ in Equation (10). 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 Equation (10), 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 U_P 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_P and Pe_{EP} respectively, in our analysis. In Figure 2(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_P 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

$$72 \frac{\coth(\sqrt{Pe_{EP}}/2)}{Pe_{EP}^{3/2}} - \frac{144}{Pe_{EP}^2} - \frac{12}{Pe_{EP}} + \frac{1}{5} \rightarrow \frac{Pe_{EP}}{210} \quad (11)$$

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

$$\frac{\delta^2}{d^2} \approx \frac{1}{Pe_{EP}} \left(1 + \frac{Pe_P^2}{210} \right) \quad (12)$$

As may be noted, Equation (12) predicts the pressure-driven cross-flow to increase the spatial variance of the analyte stream by a factor, $1 + Pe_P^2/210$, identical to that known for regular pressure-driven flow systems [35,37,38]. 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 [35,37]. Consequently, the curves depicting the ideal stream variance and the additional contribution from transverse pressure-gradients in Figure 2(a) are seen to line up parallel to each other for $Pe_{EP} \ll 1$. The value of Pe_P dictates their relative magnitude in this regime with the pressure-driven cross-flow contribution exceeding the ideal component when $Pe_P < \sqrt{210}$. In the opposite limit of $Pe_{EP} \gg 1$, the term

$$72 \frac{\coth(\sqrt{Pe_{EP}}/2)}{Pe_{EP}^{3/2}} - \frac{144}{Pe_{EP}^2} - \frac{12}{Pe_{EP}} + \frac{1}{5} \rightarrow \frac{1}{5} \quad (13)$$

yielding an expression for the stream variance given by

$$\frac{\delta^2}{d^2} \approx \frac{1}{Pe_{EP}} \left(1 + \frac{1}{5} \frac{Pe_P^2}{Pe_{EP}} \right) \quad (14)$$

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. Figure 2(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

$Pe_P < \sqrt{210}$. The value of Pe_{EP} where this cross-over occurs, denoted as Pe_{EP}^* , has been plotted in Figure 2(b) as a function of the parameter, Pe_P , in the system. The figure shows Pe_{EP}^* to approach zero when $Pe_P \rightarrow \sqrt{210}$ as expected, and increase for larger values Pe_P . In the limit of $Pe_P \gg \sqrt{210}$, the quantity $Pe_{EP}^* \approx Pe_P^2/5$ as predicted by Equation (14).

Monte Carlo simulations performed to validate the analytic results presented above provided a more comprehensive description of the sample concentration profile in the FFIEF chamber (see Figure 3). They showed that although this profile was highly asymmetric around the channel entrance for the initial condition chosen for this study (see Figure 3(b) and Figure 3(c)), it approached a symmetric distribution going downstream (see Figure 3(d)). The time scale needed for attaining the steady state value for σ^2 was reached over a period given by the larger of the diffusion and equilibration time scales, i.e. $d^2/4D$ and $W/\mu E\theta$, respectively (see Figure 4(a)). Interestingly, the spatial variance (σ^2) of the sample stream assumed its equilibrium limit given by Equation (10) at channel lengths over 2-fold shorter than that predicted by the product of U_a and these time scales in our simulations (see Figure 4(b)). Finally, some steady state results from the noted simulations have been included in Figure 5(a) and Figure 5(b) in order to validate the analytic expression for the hydrodynamic dispersion contribution from a pressure-driven cross-flow in FFIEF assays derived in the present work. In particular, these results were used to establish the dependence of the spatial variance σ^2 on the parameters Pe_P and Pe_{EP} as predicted by Equation (10) (see Figure 5(a) and Figure 5(b)). We would like to point out that an additional set of data points for a non-zero value of Pe_{EOF} , i.e. $Pe_{EOF} = 50$, has been included in Figure 5(a) to confirm that the presence of a spatially uniform transverse electroosmotic flow does not affect the quantity σ^2 although it shifts the equilibrium position of the sample stream in the FFIEF chamber as predicted by Equation (7). It must be further clarified that even though the magnitude of Pe_{EOF} does not alter the normalized stream variance in an FFIEF assay, the strength of the pH gradient in the system does. This is because the magnitude of such a gradient influences the spatial variation in the electrophoretic mobility of the analyte species and therefore the parameter Pe_{EP} . Moreover, such gradients may also introduce a significant variation in the electroosmotic flow along the channel width leading to the generation of additional pressure-driven cross-flow in the device altering the magnitude of Pe_P . Any such modification in the

value of Pe_{EP} and/or Pe_P may consequently be expected to influence the stream broadening in an FFIEF system as predicted by Equation (10).

It is also important to recognize that under experimental conditions commonly employed in an FFIEF assay, the overall spatial variance of an analyte stream can be easily dominated by the contribution from a small transverse pressure-driven flow in the system. For example, if the electrophoretic mobility gradient and the electric field strength in an FFIEF channel are chosen to be 10^{-4} cm/(Vs) and 10^3 V/cm, respectively, the parameter Pe_{EP} can be shown to attain a value of unity for a protein having a diffusion coefficient of 4×10^{-7} cm²/s in a 20 μ m deep channel. On the other hand, for a pressure-driven cross-flow velocity as small as 100 μ m/s, the quantity Pe_P acquires a magnitude of 50 for the same choice of the protein and channel depth. In this situation, Equation (10) predicts that the overall stream variance can be expected to increase by about a factor of 12.6 in the presence of the transverse pressure-gradient as compared to that estimated by the d^2/Pe_{EP} term under ideal conditions. The author would further like to reiterate that the normalized variance of the analyte stream in the presence of any steady pressure-driven cross-flow is governed by only two dimensionless parameters (Pe_{EP} and Pe_P) in an FFIEF device. In this situation, the best approach to assess the effect of the various physical operating parameters such as diffusion coefficient, channel depth, flow velocity, etc., on the overall stream variance is to first estimate their influence on the parameters Pe_{EP} and Pe_P following the definitions presented in the “Mathematical Formulation” section and then employ Equation (10) to evaluate the quantity σ^2 . Such an approach significantly simplifies the analysis and optimization of FFIEF systems establishing succinct design rules for the assay.

While the mathematical formulation described here has been applied to evaluating the spatial variance of a sample stream in an FFIEF system, it can be readily 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 equilibration and diffusion time scales, i.e. $L/U_a \gg W/\mu E\theta, d^2/4D$, where L denotes the length of the separation chamber. Moreover, our mathematical model ignores the effect of the channel sidewalls on the axial pressure-driven flow profile, and hence on the hydrodynamic dispersion component of the sample stream [38-40]. Furthermore, it does not account for any additional band broadening introduced by the abrupt changes in the channel cross-section at the entrance and/or exit of the separation chamber. It must be also noted that the mathematical model for stream broadening described in this manuscript is only applicable to systems with a stable pH gradient. In assays where this gradient fluctuates temporally due to uncontrollable factors [41,42] or is intentionally varied with time [43,44], the noted analysis may not be valid. In addition, it has been recently suggested that the production of water in isoelectric focusing system can also lead to zone broadening which again has not been accounted for in the current study [41,45]. Partial electrofocusing of analyte streams can also contribute to sample dispersion in isoelectric focusing systems but has been ignored in the present work [46-48]. In spite of these limitations, the current manuscript describes a useful approach to assessing the effect of transverse pressure-gradients in FFIEF devices and a potentially promising mathematical framework for analyzing other non-idealities often encountered in isoelectric focusing assays.

4. Conclusions

To conclude, a theoretical framework for estimating the additional broadening of analyte streams due to a transverse pressure-gradient in an FFIEF channel has been developed under laminar flow conditions. The reported framework is based on the method-of-moments approach and yields a closed-form solution for the spatial variance assuming a linear gradient in the electrophoretic mobility across the channel width. The current analysis also ignores any variation in the electroosmotic and pressure-driven cross-flows in the transverse direction which is desirable in FFIEF systems. However, it must be noted that these results can be readily extended to situations with non-zero gradients in the electroosmotic and pressure-driven cross-flow velocities provided those quantities are small compared to their electrophoretic counterpart around the equilibrium position of the analyte stream. Finally, it must be pointed out that while stream broadening in an ideal FFIEF system decreases monotonically with the applied electric field, it is no more the case in the presence of a pressure-driven cross-flow that has an electrical origin. This cross-flow may be assumed to also scale with the electric field in the assay chamber under these conditions leading to a stream broadening contribution that increases with an increase in the applied voltage across the channel width as predicted by Equation (10). Such a situation would yield an optimum value for the operating electric field in an FFIEF assay for which the zone width of the analyte stream will be minimized.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- sample concentration moments in FFIEF with a pressure-driven cross-flow is analyzed
- an analytical expression for describing hydrodynamic stream broadening is derived
- normalized stream variance is determined by two dimensionless Péclet numbers
- small lateral pressure-driven flows can even dominate stream broadening in FFIEF

Figure 1. (a) Schematic of an FFIEF fractionation process with a pressure-driven cross-flow in the channel as described in this article. (b) Top view of an FFIEF device relevant to the mathematical analysis presented in this work.

Figure 2.

(a) Dependence of the equilibrium stream variance on the quantity Pe_{EP} as predicted by Equation (10) in the presence of a steady pressure-driven cross-flow in an FFIEF system. “Ideal conditions” here correspond to the situation when $Pe_P = 0$. **(b)** Variation in the value of Pe_{EP}^* as function of Pe_P in an FFIEF assay with a steady pressure-driven cross-flow. The quantity Pe_{EP}^* here denotes the value of at which the additional spatial variance from the pressure-driven cross-flow equals that expected under ideal conditions, i.e. d^2/Pe_{EP} .

Figure 3.

(a) Spatial distribution of analyte molecules in an FFIEF chamber at steady state as estimated based on a Monte Carlo simulation performed for a choice of $Pe_{EP} = 50$ and $Pe_P = 26.67$. Steady state distribution of analyte molecules along the width of the FFZE chamber at (b) $Z^* = 1$ (c) $Z^* = 3$ and (d) $Z^* = 56$. Notice that the symmetry in the molecular distribution improves downstream of the channel inlet, i.e. with increase in the value of Z^* .

Figure 4.

(a) Temporal evolution of the normalized stream variance at $Z^* = 3$ and 56 as predicted by our Monte Carlo simulations performed for a choice of $Pe_{EP} = 50$ and $Pe_P = 26.67$. The sub-figure shows that σ^2 practically attains its steady-state value when $t > 3W/\mu E\theta$ establishing a time scale for the equilibration of the solute distribution in the FFIEF channel. Note that the steady state stream variance in the vicinity of the channel entrance is always smaller than that predicted by Equation (10). (b) Spatial evolution of the normalized stream variance with Z^* at a normalized time of $\mu E\theta t/W = 6$ again performed for a choice of $Pe_{EP} = 50$ and $Pe_P = 26.67$. The sub-figure shows that the steady state stream variance assumes the value predicted by Equation (10) only when $Z^* > U_a W/\mu E\theta$ establishing a length scale for the validity of this mathematical expression. The dotted lines in both sub-figures correspond to the steady state value predicted by Equation (10).

Figure 5.

(a) Variation in the normalized stream variance as a function of the Pe_P as predicted by Equation (10) (solid lines) and our Monte Carlo simulations (solid symbols) (b) Variation in the normalized stream variance as a function of the Pe_{EP} as predicted by Equation (10) (solid lines) and our Monte Carlo simulations (solid symbols). The error bars associated with the Monte Carlo simulation results were estimated based on 5 independent runs.