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Joule Heating Induced Stream Broadening in Free-Flow Zone Electrophoresis

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Abstract

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. In this article, an analytic theory is presented to quantitate such effects of Joule heating on FFZE assays in the limit of small temperature differentials across the channel gap yielding a closed form expression for the stream position and zone variance under equilibrium conditions. A method-of-moments approach is employed to develop this analytic theory, which is further validated with numerical solutions of the governing equations. Interestingly, the noted analyses predict that Joule heating can drift the location of the analyte stream either way of its equilibrium position realized in the absence of any temperature rise in the system, and also tends to reduce zone dispersion. The extent of these modifications, however, is governed by the electric field induced temperature rise and three Péclet numbers evaluated based on the axial pressure-driven flow, transverse electroosmotic and electrophoretic solute velocities in the separation chamber. Monte Carlo simulations of the FFZE system further establish a time and a length scale over which the results from the analytic theory are valid.

Keywords

band broadening; free-flow zone electrophoresis; Joule heating; mathematical modeling; method-of-moments

1. Introduction

The ability to continuously separate out a target sample fraction from a complex mixture is desirable in assays 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 [1–3]. Free-flow zone electrophoresis (FFZE) offers a useful approach in this regard enabling the extraction of analytes based on their

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electrophoretic mobilities, and is applicable to a wide range of chemical and biological samples [4]. Moreover, the use of relatively gentle operating conditions in this technique combined with its high throughput makes it a particularly valuable tool for simplifying the sample feed to an electrophoretic or chromatographic system. The FFZE assay typically relies 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, the analyte molecules are deflected from their original path based on their electrophoretic mobilities causing them to exit the FFZE chamber at different lateral positions. The resolution of the separation process consequently, is determined by the differences in the electrophoretic transport rates of the solute species as well as the amount of band dispersion they undergo during their transit through the separation column [5].

It has been previously shown in the literature that under ideal conditions the spatial variance (σ^2) of an analyte stream at the outlet position of an FFZE device may be expressed as [6,7]

$$\sigma^2 = \underbrace{\frac{2DL}{U_P}}_{\text{diffusive broadening}} + \underbrace{\frac{2(\mu_{EOF} + \mu_{EP})^2 E^2 DL}{U_P^3}}_{\text{orientation factor}} + \underbrace{\frac{1}{105} \frac{(\mu_{EOF} + \mu_{EP})^2 E^2 d^2 L (\mu_{EOF} + \mu_{EP})^2 E^2 d^2 L}{U_P D}}_{\text{hydrodynamic broadening}} + \underbrace{\frac{b^2}{12}}_{\text{injection variance}} \quad (1)$$

In the above expression, the symbols D , U_P , μ_{EOF} and μ_{EP} refer to the molecular diffusion coefficient, spatially averaged axial pressure-driven velocity, electroosmotic mobility of the fluid and electrophoretic mobility of the analyte species, respectively. In addition, the characters E , b , d and L in the equation represent the transverse electric field, the width of the sample stream at the channel entrance, the depth and the length of the FFZE compartment, respectively. Equation (1) shows that the overall stream variance in an FFZE system ideally comprises four additive contributions [7]. These include the diffusive and hydrodynamic broadening components, a contribution from the injection width and an orientation factor term, which accounts for the fact that the spatial variance of the analyte stream is not measured orthogonal to its flow direction. Besides these contributions, the electric field required to actuate the assay also has a tendency to generate heat (Joule heating) in the system which modifies analyte transport from that realized in the absence of Joule heating [8,9]. In general, such electrical heating raises the liquid temperature at the center of the separation chamber compared to that around the walls producing a variation in the equilibrium position of the analyte molecules across the channel gap. For small temperature differentials across the channel gap, this variation predominantly arises due to

the temperature dependence of liquid viscosity and analyte diffusivity yielding a faster flow and diffusion of the solute species around the channel center. In this article, a mathematical description of such Joule heating induced modifications in analyte transport has been presented for small temperature differentials across the channel gap. A method-of-moments approach [10] has been taken to solve the relevant material balance equations yielding a closed-form expression for the stream position and zone variance under equilibrium conditions. The results from the analytic theory have been later validated with numerical simulations that take into account the higher order effects of the temperature gradient on the separation performance. Finally, Monte Carlo simulations of the FFZE system have been performed to establish a time and length scale over which the predicted stream width is attained in the presence of Joule heating.

2. Mathematical Formulation

To evaluate the effect of Joule heating on solutal transport in an FFZE system, the present analysis considers the flow of an analyte stream between two parallel plates separated by a distance d (see Figure 1). A pressure-gradient applied along the axial direction (z -coordinate) drives this sample flow in the presence of an electric field (E) imposed across the width of the separation chamber (along the x -coordinate). In order to simplify the present analysis, the locations of the parallel plates are assumed to be $y = \pm d/2$ producing a liquid flow and analyte concentration profile that is symmetric with respect to the y -coordinate. 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

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

where T_0 refers to the temperature at the parallel plates and the quantities k and λ denote the ionic and thermal conductivity of the background electrolyte, respectively. It is important to point out that although the conductivity parameters k and λ in Equation (2) 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% [11–15]. In this situation, the leading order effect of Joule heating may be captured by assuming k to be spatially constant ($0.6 \text{ Wm}^{-1}\text{K}^{-1}$ 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 $\lambda_0 = 0.14 \text{ m}^2 \text{ S mol}^{-1}$ (for aqueous KCl solutions), $\alpha = 0.025 \text{ K}^{-1}$ (for water) and I_0 equals the ion concentration in the background electrolyte in mol m^{-3} [12–15].

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 [16, 17]. 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 [18]. 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 Equation (2).

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

$$\frac{d^2\theta}{dy^{*2}} = -(1 + \alpha \Delta T \theta) \quad \text{boundary conditions: } \theta = 0 \text{ at } y^* = \pm \frac{1}{2} \quad (3)$$

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 $y^* = y/d$. It is well known that Equation (3) admits a closed-form solution [18,19] which for small values of ΔT , predicts θ to vary quadratically with y^* as noted below

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

Equation (4) 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 Figure 2(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 μm and 10 – 25 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_t) to comprise two additive contributions from electroosmosis and electrophoresis, i.e., $u_t = \mu_{EP}E + \mu_{EOF}E$. Interestingly however, while the non-uniformity in liquid temperature described by Equation (4) 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 [20]. In this situation, the spatial variation in the quantity u_t may be expressed as

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

$$\eta = A \exp\left(\frac{B}{T}\right) = \eta_0 \left(1 - \frac{B\Delta T\theta}{T_0^2}\right) = \eta_0 \left[1 - \frac{B\Delta T(1 - 4y^{*2})}{8T_0^2 \cos(\sqrt{\alpha\Delta T}/2)}\right] + O(\Delta T^2) \quad (5)$$

where $A = 2.76 \times 10^{-6}$ Pa s, $B = 1713$ K and $\eta_0 = A \exp(B/T_0)$ [21]. These considerations lead to an advection-diffusion equation governing the analyte concentration (C) in the assay chamber as follows

$$\mu_{EP}^0 E \frac{\eta_0}{\eta} \frac{\partial C}{\partial x} + \mu_{EOF}^0 E \frac{\partial C}{\partial x} + u_p \frac{\partial C}{\partial z} = D \frac{\partial^2 C}{\partial x^2} + \frac{\partial}{\partial y} \left(D \frac{\partial C}{\partial y} \right) + D \frac{\partial^2 C}{\partial z^2} \quad (6)$$

where u_p and D denote the axial pressure-driven liquid velocity and analyte diffusion coefficient, respectively, which are both functions of temperature and therefore the y -coordinate in the system. The spatial variation in u_p may be determined by solving the Navier-Stokes equation while accounting for the temperature dependence of liquid viscosity yielding $u_p = U_p^0 \left[3(1 - 4y^{*2})/2 + 3\beta(1 - 4y^{*2})^2/4 \right]$ to the leading order in ΔT where U_p^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. Now upon normalizing all length scales with respect to d , i.e., $x^*, y^*, z^* = x/d, y/d, z/d$ and the sample concentration by its inlet value (C_0), i.e., $C^* = C/C_0$, Equation (6) may be expressed as

$$Pe_{EP}f\frac{\partial C^*}{\partial x^*} + Pe_{EOF}\frac{\partial C^*}{\partial x^*} + Pe_Pg\frac{\partial C^*}{\partial z^*} = h\frac{\partial^2 C^*}{\partial x^{*2}} + \frac{\partial}{\partial y^*}\left(h\frac{\partial C^*}{\partial y^*}\right) + h\frac{\partial^2 C^*}{\partial z^{*2}} \quad (7)$$

where the functions $f = \eta_0/\eta = 1 + \beta(1 - 4y^{*2})$, $g = u_P/U_P^0 = 3(1 - 4y^{*2})/2 + 3\beta(1 - 4y^{*2})^2/4$ 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 ΔT . The various parameters relevant to these functions are defined as $\beta = B\Delta T/\{8T_0^2\cos(\sqrt{\alpha\Delta T}/2)\}$, $\gamma = \Delta T(1 + B/T_0)/\{8T_0\cos(\sqrt{\alpha\Delta T}/2)\}$, $U_P^0 = \Delta P d^2/(12\eta_0 L)$ and $D_0 = k_B T_0/(6\pi\eta_0 R)$ with ΔP 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 Equation (7) 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 $Pe_P = U_P^0 d/D_0$, 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. As may be noted, Equation (7) 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 ΔT . In this situation, it is important to have a sense of the magnitude of these quantities which have been included in Figure 2(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.

Finally, the boundary conditions relevant to Equation (7) may be written as $\partial C^*/\partial y^* = 0$ at $y^* = \pm 1/2$ and $C^*, \partial C^*/\partial x^* = 0$ as $x^* \rightarrow \pm \infty$ in order to then seek a solution to the dimensionless analyte concentration in the system. In addition to these constraints, the amount of analyte flowing per unit time through any x - y plane is also assumed to be a constant (say M) in the current analysis which can be equated to the integral

$$\left(3C_0U_P^0d^2/2\right)\int_{-1/2}^{1/2}\int_{-\infty}^{\infty}(1-4y^{*2})C^*dx^*dy^*$$

to satisfy the material balance for the analyte species. Furthermore, this constant may be expressed as $M = C_0U_P^0bd$ based on the uniform distribution of the solute molecules across a width b at the entrance of the FFZE channel ($z^* = 0$). The dimensionless diffusion-advection equation discussed above has been solved in

the present work using the method-of-moments approach. To this end, Equation (7) was first multiplied by x^{*n} and then integrated along the x^* -coordinate from $-\infty$ to ∞ to yield [7,10,22,23]

$$-nPe_{EP}f\phi_{n-1} - nPe_{EOF}\phi_{n-1} + Pe_Pg\frac{\partial\phi_n}{\partial z^*} = n(n-1)h\phi_{n-2} + \frac{\partial}{\partial y^*}\left(h\frac{\partial\phi_n}{\partial y^*}\right) + h\frac{\partial^2 C^*}{\partial z^{*2}} \quad (8)$$

$$\left.\frac{\partial\phi_n}{\partial y^*}\right|_{y^*=\pm 1/2} = 0; \quad \int_{-1/2}^{1/2} (1-4y^{*2})\phi_0 dy^* = \frac{2M}{3C_0U_P^0d^2} = \frac{2b}{3d} = \frac{2}{3}\delta$$

$$\text{where } \phi_n = \int_{-\infty}^{\infty} x^{*n} C^* dx^*.$$

Later, Equation (8) was integrated along the y^* -coordinate over the region between the parallel plates to obtain

$$\begin{aligned} & -nPe_{EP} \int_{-1/2}^{1/2} f\phi_{n-1} dy^* - nPe_{EOF}m_{n-1} + Pe_P \int_{-1/2}^{1/2} \left(g\frac{\partial\phi_n}{\partial z^*}\right) dy^* \quad (9) \\ & = n(n-1) \int_{-1/2}^{1/2} h\phi_{n-2} dy^* + \int_{-1/2}^{1/2} \left(h\frac{\partial^2\phi_n}{\partial z^{*2}}\right) dy^* \end{aligned}$$

$$\text{where } m_n = \int_{-1/2}^{1/2} \phi_n dy^*.$$

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.

Besides capturing the leading order effect of Joule heating on sample transport using the mathematical formulation described above, the concentration-moment equations were also solved using numerical techniques without linearizing the temperature dependence of η and D on ΔT . These concentration-moment equations were again derived from Equation (7) but

now expressing $\eta = A \exp(B/T)$, $D = k_B T / (6\pi\eta R)$ and temperature profile using the non-linearized form of Equation (4). The resulting mathematical expressions similar to those included in Equations (8) and (9) were later solved using in-house written MATLAB codes for establishing a value of the characteristic temperature rise (ΔT) beyond which the linearized solutions to the concentration-moment equations become error-prone. These numerical results were further validated using Monte Carlo simulations, which also provided a length and a time scale over which the predictions of Equations (8) and (9) are applicable. For the noted simulations, the temperature profile across the parallel plates was again assumed to be given by Equation (4) without linearizing it with respect to ΔT . In addition, the spatial variation in the viscosity of the background electrolyte and the diffusion coefficient of the analyte molecules were accounted for using the correlations $\eta = A \exp(B/T)$ and $D = k_B T / (6\pi\eta R)$, respectively, also without linearizing them with respect to the characteristic temperature rise. 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 $\vec{u} = u_t \hat{e}_x + u_p \hat{e}_z$ 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 $\sqrt{2D\Delta t}$. 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 with the domain extending 200 units in the axial pressure-driven flow direction ($0 \leq z^* \leq 200$). This FFZE chamber was assumed to be unbounded along the x -coordinate to eliminate the effect of the channel sidewalls on stream dispersion. 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 FFZE chamber. In this situation, the steady state variance of the analyte stream was expected to be dominated by the hydrodynamic dispersion term for large values of $Pe_{EP} + Pe_{EOF}$, i.e., $Pe_{EP} + Pe_{EOF} \gg \sqrt{105}$. It was also ensured that the stream width at the channel outlet reached its equilibrium value by choosing the length of the FFZE chamber to be at least 8 times larger than the product of the axial stream velocity (U_p^0) and the characteristic diffusion time scale ($d^2/4D_0$) in the system, i.e., $L \geq 2U_p^0 d^2/D_0$. Finally, these simulations were initiated by assuming a Gaussian distribution of the analyte molecules around the center of mass of the sample stream expected at steady state with a spatial variance equal to that of the injected stream, and therefore, negligibly small compared to its equilibrium value. A constant analyte flow was maintained in the system by re-introducing all molecules exiting the FFZE chamber at $z^* = 200$ 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 and diffusion time scales in the system, i.e., L/U_p^0 and $d^2/4D_0$, respectively. All Monte Carlo simulations included in this study were again performed using in-house codes written in MATLAB.

3. Results and Discussion

The integral boundary condition in Equation (8) 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 $d(hd\phi_0/dy^*)/dy^* = 0$ yielding the solutions $\phi_0 = m_0 = \delta$. The corresponding equation for ϕ_1 in this situation becomes

$$Pe_P g \frac{\partial \phi_1}{\partial z^*} - h \frac{\partial^2 \phi_1}{\partial z^{*2}} - \frac{\partial}{\partial y^*} \left(h \frac{\partial \phi_1}{\partial y^*} \right) = Pe_{EP} f \delta + Pe_{EOF} \delta \quad (10)$$

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 Equation (10) and later matching the coefficients of z^* , it is possible to show that $d(hd\psi_{11}/dy^*)/dy^* = 0$ which admits the following solution to the leading order in ΔT

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

It must be noted that the parameters β and γ both scale with the quantity ΔT 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 Equation (10) are equated to obtain $d(hd\psi_{10}/dy^*)/dy^* = Pe_P g \psi_{11} - Pe_{EP} f \delta - Pe_{EOF} \delta$ which is solved to the leading order in ΔT yielding

$$\begin{aligned} \psi_{10} = (Pe_{EP} + Pe_{EOF})\delta & \left[\frac{(120y^{*2} - 240y^{*4} - 7)}{480} \right. \\ & \left. + \left(\frac{\beta}{5600} - \frac{\gamma}{1680} \right) (420y^{*2} - 1680y^{*4} + 2240y^{*6} - 19) \right] \end{aligned} \quad (12)$$

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

$$\frac{m_1}{m_0} = \left[\frac{(Pe_{EP} + Pe_{EOF})}{Pe_P} + \frac{2\beta(2Pe_{EP} - 3Pe_{EOF})}{15Pe_P} \right] z^* \quad (13)$$

which shows Joule heating to shift this quantity by an additional term proportional to β . Moreover, Equation (13) 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 (ΔP) 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 Figure 3(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 Figure 3(b). It must be noted that while the solid curves in Figure 3(a)/(b) correspond to the predictions of the analytic theory, the dashed ones represent simulation results obtained by numerically solving Equation (10) without linearizing the expressions for the liquid viscosity, analyte diffusivity and the temperature profile across the channel gap with respect to ΔT . As expected, the deviation between the two curves is seen to grow for larger values of ΔT with the analytic theory underpredicting the numerical simulations for the case of no electrophoresis. Nevertheless, this mismatch is still below 0.2% for $\beta \leq 0.1$ and the operating conditions considered in Figure 3(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 Figure 3(b).

Now for estimating the second moment of C^* , Equation (8) is again referred back to arrive at the differential equation governing ϕ_2

$$Pe_P g \frac{\partial \phi_2}{\partial z^*} - h \frac{\partial^2 \phi_2}{\partial z^{*2}} - \frac{\partial}{\partial y^*} \left(h \frac{\partial \phi_2}{\partial y^*} \right) = 2Pe_{EP} f \phi_1 + 2Pe_{EOF} \phi_1 + 2h\delta \quad (14)$$

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 (14) and later matching the coefficients for z^{*2} , it can be shown that $d(hd\psi_{22}/dy^*)/dy^* = 0$ which admits the following solution to the leading order in ΔT

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

Later matching the coefficients for z^* in Equation (14) yields

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

$$\begin{aligned} \psi_{21} = & \frac{(Pe_{EP} + Pe_{EOF})^2 \delta (280y^{*2} - 560y^{*4} - 11)}{560Pe_P} + \frac{2\delta}{Pe_P} \left[1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} \right] \quad (16) \\ & + \frac{(Pe_{EP} + Pe_{EOF})\delta\beta}{25200Pe_P} \left[Pe_{EP}(20160y^{*6} - 21840y^{*4} + 7140y^{*2} - 367) \right. \\ & \left. + Pe_{EOF}(20160y^{*6} - 5040y^{*4} - 1260y^{*2} + 123) \right] \\ & + \frac{(Pe_{EP} + Pe_{EOF})^2 \delta\gamma (5040y^{*4} - 6720y^{*6} - 1260y^{*2} + 41)}{2520Pe_P} - \frac{4\delta\beta}{5Pe_P} \\ & + \frac{4\delta\gamma}{3Pe_P} \left[1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} \right] + \frac{4(Pe_{EP} + Pe_{EOF})\delta\beta(Pe_{EP} - 9Pe_{EOF})}{15Pe_P^3} \end{aligned}$$

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 Equation (14).

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

$\delta^3/12$ 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 Equations (15) and (16) over the channel gap, the second moment of C^* in any $x - y$ plane may be estimated as

$$\begin{aligned}
m_2 = & \frac{(Pe_{EP} + Pe_{EOF})^2 \delta}{Pe_P^2} \left[1 + \frac{4(2Pe_{EP} - 3Pe_{EOF})\beta}{15(Pe_{EP} + Pe_{EOF})} \right] z^*{}^2 \quad (17) \\
& + \left[\frac{2\delta}{Pe_P} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} + \right. \\
& + \frac{4\delta\gamma}{3Pe_P} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} - \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \\
& \left. + \frac{4\delta\beta(Pe_{EP} + Pe_{EOF})(Pe_{EP} - 9Pe_{EOF})}{15Pe_P^3} - \frac{4\delta\beta}{5Pe_P} \right] z^* + \frac{\delta^3}{12}
\end{aligned}$$

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

$$\begin{aligned}
\sigma^*{}^2 = \frac{\sigma^2}{d^2} = & \left[\frac{2}{Pe_P} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \right. \quad (18) \\
& + \frac{4\beta}{5Pe_P} \left\{ \frac{(Pe_{EP} + Pe_{EOF})(Pe_{EP} - 9Pe_{EOF})}{3Pe_P^2} - 1 \right\} \\
& \left. + \frac{4\gamma}{3Pe_P} \left\{ 1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} - \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right\} \right] z^* + \frac{\delta^2}{12}
\end{aligned}$$

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. Equation (18) 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 ΔT . 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. Figure (4) 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.,

$Pe_P, Pe_{EP} + Pe_{EOF} \gg \sqrt{210}$. 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 $t = z^*d(1 - 2\beta/5)/U_P^0$ denotes the residence time of the analyte stream in the FFZE chamber. Upon incorporating this information in Equation (18) and making the relevant rearrangement, it is now possible to express

$$\begin{aligned}
 K = D_0 & \left[1 + \left(\frac{Pe_{EP} + Pe_{EOF}}{Pe_P} \right)^2 + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right. \\
 & + \frac{2\beta}{5} \left[\frac{2(Pe_{EP} + Pe_{EOF})(2Pe_{EP} - 3Pe_{EOF})}{3Pe_P^2} + \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right] \\
 & \left. + \frac{2\gamma}{3} \left[1 + \frac{(Pe_{EP} + Pe_{EOF})^2}{Pe_P^2} - \frac{(Pe_{EP} + Pe_{EOF})^2}{210} \right] \right] \quad (19)
 \end{aligned}$$

Equation (19) 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

$4\beta D_0 (Pe_{EP} + Pe_{EOF})(2Pe_{EP} - 3Pe_{EOF}) / (15Pe_P^2)$ exactly accounts for the change in the orientation factor to the leading order in ΔT based on the drift in the lateral equilibrium stream position predicted by Equation (13). The other term scaling with β in Equation (19), 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 Equation (19) 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 $2\gamma D_0 \left[1 + (Pe_{EP} + Pe_{EOF})^2 / Pe_P^2 \right] / 3$. 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 Figure 2(b)). Figure (4) also shows that Joule heating can be expected to reduce stream broadening by about 20% in the absence of electrophoresis under conditions when $Pe_P = 1000$ 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_P 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 $U_P^0 = 1 \text{ cm/s}$, $E = 8 \text{ kV/cm}$ and μ_{EP}^0 or $\mu_{EOF}^0 = 3 \times 10^{-4} \text{ cm}^2/\text{Vs}$.

The analytic and numerical results presented above were further validated with Monte Carlo simulations which provided a more comprehensive description of the sample concentration profile in the FFZE chamber in the presence of Joule heating (see Figure 5). These simulations showed that for the initial condition chosen in this study, the solute distribution

starts asymmetric around the center of mass of the analyte stream in the vicinity of the channel entrance (see Figure 5(c) and 5(d)) but becomes increasingly symmetric going downstream (see Figure 5(a)). The time scale needed for attaining the steady state value for σ^2 was reached over a period given by the diffusion time scale across the channel depth, i.e., $d^2/4D$ (see Figure 6(a)). Interestingly however, the spatial variance (σ^2) of the sample stream throughout the FFZE chamber was well described by the Taylor–Aris dispersion limit determined by the numerical solution of the governing equations (see Figure 6(b)). Finally, some steady state results from the Monte Carlo simulations have been included in Figure (7) in order to validate the analytic/numerical results for the hydrodynamic dispersion contribution from Joule heating in FFZE assays. In particular, these results were used to establish the dependence of the spatial variance σ^2 on the parameter Pe_p as predicted by Equation (10) (see Figure 7).

Notice that the mathematical formulation outlined in equations 1 through 7 for estimating the Taylor-Aris dispersion of sample streams relies on the decoupling of the energy, momentum and mass conservation equations. Although this approach reduces the computational effort involved in deriving the desired information significantly, it yields accurate results only when some simplifying assumptions are satisfied. For example, the thermal profile in a straight microchamber may be calculated independently of the velocity and solute concentration fields provided the temperature gradient along the x - and z -coordinates and the advective velocity along the y -coordinate may be neglected. While the axial temperature gradients (along the z -coordinate) are minimized in a fully-developed thermal profile, thermal energy transport across the channel width (x -coordinate) 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 as is the case in this analysis. Moreover, 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). Interestingly, these two assumptions are generally satisfied in the central section of long and wide FFZE chambers, which justifies the ignorance of the advective terms and the diffusive term along the x - and z -directions in the energy equation, i.e., Equation (1). Moreover, the decoupling of the energy and momentum conservation expressions from the mass conservation equation is known to be strictly valid for dilute samples for which the solute concentration profile does not influence any of the material properties in the system. Furthermore, the Taylor-Aris dispersion limit for band broadening is applicable to situations only when the transit time for the sample species through the microchannel is significantly larger than their diffusion time across the channel cross-section, i.e., $L/U \gg d^2/D_0$. These conditions again are easily met in 10-100 μm deep FFZE chambers that are longer and wider than a few millimeters carrying dilute samples of small molecules with D_0 values in the range of 10^{-5} - 10^{-6} cm^2/s . To substantiate our arguments on the validity of the assumptions made in our simplified model, we also carried out some full blown 3-D simulations of the concerned problem using the commercially available COMSOL package and have compared these results to those discussed in the previous paragraph. In these simulations, the energy, momentum and mass conservation equations were solved in a coupled manner within the FFZE chamber of rectangular cross-

section that was chosen to be 25 μm deep, 2 mm wide and 1 cm long. An infinitesimally narrow sample stream was introduced at the entrance of this chamber midway across its width, and the equilibrium spatial variance (σ^2) of the stream evaluated along the channel axis during its migration through the compartment. The Taylor-Aris dispersivity K was later estimated from the spatial derivative of σ^2 as $K = (D_0/2)(d\sigma^2/dz)$ to enable its comparison with the results from the simpler analytical/numerical models. As may be seen from the data included in Figure 6(b), the COMSOL data matched closely with the simplified numerical and Monte Carlo simulations establishing the validity of our assumptions in question.

Finally, it must be pointed out that while the mathematical formulation described here has been applied to evaluating the spatial variance of a sample stream due to Joule heating in an FFZE 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 diffusion time scale, i.e., $L/U_p^0 \gg d^2/4D$. Moreover, the present 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 [24]. 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 FFZE systems rendering the current mathematical model highly useful in assessing the performance of free-flow zone electrophoretic separations.

4. Conclusions

In summary, the present work outlines a useful theoretical framework for quantitating stream dispersion in FFZE systems with Joule heating effects. It predicts that the higher temperature at the channel center under these conditions can drift the center of mass of the sample zone either way of its equilibrium position realized in the absence of Joule heating. In addition, the hydrodynamic contribution to the stream width can be significantly reduced by such thermal effects due to faster equilibration of the analyte molecules across the different flow streamlines. The present analysis however, ignores the presence of any axial temperature gradients in an FFZE system which has been shown to introduce additional dispersion of sample zones but only for channels that are at most a few millimeters long [16,17]. Fortunately, the results from the simplified model included in this manuscript are applicable to most microchip based FFZE chambers which typically have widths on the order of millimeters and lengths on the order of centimeters.

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Figure 1.

(a) Schematic of the FFZE fractionation process as described in this article. (b) Top view of a FFZE device relevant to the mathematical analysis presented in this work.

Figure 2.

(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 .

Figure 3. 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.

Figure 4.

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 $\sigma^* = \sigma/d$.

Figure 5.

(a) Spatial distribution of the analyte molecules in an FFZE chamber at steady state as estimated based on a Monte Carlo simulation performed for a choice of $Pe_{EP}=200$, $Pe_{EOF}=0$ and $Pe_P=100$. Steady state distribution of analyte molecules along the width of the FFZE chamber at (b) $z^*=170$ (c) $z^*=30$ and (d) $z^*=10$. Notice that the symmetry in the molecular distribution about the center of mass of the analyte stream improves downstream of the channel inlet, i.e., with increase in z^* .

Figure 6.

(a) Temporal evolution of the normalized stream variance as predicted by our Monte Carlo simulation performed for a choice of $Pe_{EP}=200$ and $Pe_P=100$. (b) Spatial evolution of the stream variance downstream of the sample inlet, i.e., $z=0$, at a normalized time of $t^*=2.5$ again performed for a choice of $Pe_{EP}=200$ and $Pe_P=100$. The solid circles in the sub-figure represent the estimated values of the normalized stream variance based on COMSOL simulations. The energy, momentum and mass conservation equations were solved in a coupled manner in these simulations in order to validate the underlying assumptions of the simplified mathematical model described in this work for analyzing Joule heating effects in FFZE systems.

Figure 7. Comparison of our Monte Carlo simulation results to the theoretical predictions of the mathematical models outlined in this work.