

# Band Broadening in a Microcapillary with a Stepwise Change in the $\zeta$ -potential

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**In capillary zone electrophoresis (CZE), adsorption of charged analytes to walls has been observed to cause significant band broadening. The effect is believed to be due to modification of the flow pattern and the consequent Taylor dispersion caused by the alteration of the wall charge by the adsorbed analytes. Experiments using neutral (nonadsorbing) analytes in capillaries in which the  $\zeta$ -potential has been deliberately altered in a controlled way by chemically coating a segment of the capillary have been performed by Towns and Regnier (*Anal. Chem.* 1992, 64, 2473) in an effort to understand the mechanism of the band broadening. In this paper, the Taylor dispersion due to electroosmotic flow in such a partially coated capillary is calculated and compared to the experimental data. The theoretical predictions are found to be in excellent agreement with the data, thus supporting the hypothesis that the decreased resolution can be attributed to Taylor dispersion due to the induced pressure gradient brought about by the nonuniformity of the  $\zeta$ -potential.**

Capillary zone electrophoresis (CZE) has gained popularity over the past decade as the method of choice for analyzing biomolecules by exploiting their different electrophoretic mobilities in aqueous solution.<sup>1,2</sup> In its simplest form, the CZE apparatus consists of a microcapillary mounted between two reservoirs and filled with an ionic buffer at the desired pH. An electric potential difference is applied between the inlet and outlet reservoirs by means of electrodes. The sample is introduced as a plug near the inlet and is allowed to migrate toward the outlet as a result of the electroosmotic flow induced by the electric field. The velocity of migration of any particular species of molecules is the vector sum of this induced electroosmotic flow (EOF) velocity<sup>3</sup> and the electrophoretic migration velocity of the species in the absence of bulk flow. As a result, the analyte separates into bands, each representing a different species. The arrival time of each of these bands at a fixed detector is measured and used to identify sample components.

The figure of merit for an electrophoretic system is its resolution, often expressed as the number of theoretical plates,

$N$ , defined as  $N = X^2/\sigma^2$ , where  $X$  is the distance between the injection point and the detector, and,  $\sigma^2$  is the variance of the analyte concentration,  $c$ , at the detector, defined as  $\sigma^2 = \langle \bar{c}^2 \rangle - \langle \bar{c} \rangle^2$ , where the overbar denotes average over the cross-section, and,  $\langle \rangle$  denotes average in the axial direction. In CZE, one tries to make  $N$  as large as possible. A theoretical upperbound is  $N < N_{\max} = (u_e/2D)X$ , where  $u_e$  is the algebraic sum of the electroosmotic and electrophoretic migration velocity, and  $D$  is the molecular diffusion coefficient of the species. In practice, there are various sources of band broadening<sup>4–6</sup> that prevent  $N$  from reaching this maximum theoretically allowed value of  $N_{\max}$ . One such source of band broadening is the tendency of certain analytes, particularly proteins, to stick to the walls of the capillary, primarily because of electrostatic attraction.

The time-dependent advection–diffusion problem in which the adsorptive flux of analyte at the channel wall is a linear function of the local value of the concentration has been studied by Štědrý et al.<sup>7</sup> It is shown that the adsorption leads to an increase in the effective diffusion coefficient (defined as the slope of the variance as a function of time) as well as a small time-independent downward shift in the variance. The former effect is dominant in most practical situations so that the variance is usually increased. However, the above analysis does not take into account the flow modification due to any changes in the  $\zeta$ -potential brought about by adsorption of charged species. This latter effect could be a significant source of band broadening.

Unlike the classical (pressure driven) Poiseuille flow, which has a parabolic velocity profile, a “pure” EOF (with no imposed pressure gradient) has a uniform velocity over the capillary cross section except within a thin Debye layer at the walls. Since a uniform flow profile has no Taylor dispersion associated with it,  $N$  can attain values very close to  $N_{\max}$ . The existence of a thin Debye layer ( $\sim 10$  nm) adjacent to the wall does indeed result in some dispersion;<sup>8,9</sup> however, since the channel width (typically 20–200  $\mu\text{m}$ ) is much larger than the Debye layer thickness, this contribution is usually very small. If, however, the adsorption of charged analytes to the capillary wall changes the  $\zeta$ -potential<sup>3</sup> in

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a nonuniform manner along the channel,<sup>10</sup> the requirement of mass continuity forces the appearance of an axial pressure gradient. The flow profile is no longer uniform, and the resultant Taylor dispersion could lead to a rapid increase in the variance of the analyte concentration.

“Taylor dispersion” refers to the axial dispersion of solutes in a fluid stream due to the simultaneous action of shear and diffusion. The phenomenon appears to have been first observed experimentally by Griffiths,<sup>11</sup> though a theoretical understanding of the underlying mechanism is due to G. I. Taylor.<sup>12</sup> Taylor discovered a remarkably simple description for the problem of dispersion of solutes in fluids flowing through narrow tubes. Subsequently, these results were further clarified and extended by Aris.<sup>13</sup> They showed that after sufficient time has elapsed since the injection of the solute plug, the mean (averaged over the cross section) solute concentration may be described by a Gaussian function of the distance along the tube axis. The peak of this Gaussian is advected at the speed of the mean flow, and its width increases in time as if the mean concentration had a certain “effective diffusion” coefficient for which a simple analytical formula could be provided. Physically, this effective diffusion coefficient is the consequence of the simultaneous action of shear and diffusion. These ideas have since found wide practical application and have come to be known as “Taylor dispersion”, “Taylor–Aris dispersion” or “Shear-induced dispersion”.

The problem of EOF through a microfluidic channel with variable wall  $\zeta$ -potential has been studied recently by several authors. Anderson and Idol<sup>14</sup> considered the problem of EOF through a uniform, infinite, straight cylindrical capillary with a  $\zeta$ -potential that varies solely in the axial direction. An exact solution to the Stokes equation for this problem was obtained in the limit of infinitely thin Debye layers. The solution predicted the expected induced pressure gradient fluctuations and the resulting nonuniformity in the flow profile across the channel cross-section. A simpler special case of the general problem considered by Anderson and Idol is that of flow through a cylindrical capillary with a wall charge that undergoes a stepwise change in the axial direction. This problem was considered by Herr et al.<sup>15</sup> They showed by means of a simple calculation that the requirement of mass continuity forces the appearance of a pressure gradient and associated Poiseuille flow in the capillary. The prediction was checked experimentally by joining two capillaries with different wall  $\zeta$ -potentials to produce a single capillary with a stepwise variation in  $\zeta$ -potential. The flow profile was measured using a caged fluorescence technique and compared with the theoretical calculation. Excellent agreement was obtained. Band broadening due to increased axial dispersion was observed in these experiments, but quantitative measurements of  $N$  were not attempted. The effect of analyte adsorption on the mean flow speed as well as the resolution in CZE was studied by Towns and Regnier<sup>10</sup> through a set of carefully controlled experiments. They found that for negatively charged fused-silica capillaries, the amount of analyte lost to adsorption, the elution time, and the dispersion

increased with the degree of positive charge on the proteins as measured by their isoelectric point. All of these quantities could be reduced by reversing the wall charge by applying a synthetic coating of poly(ethylenimine) (PEI 200). To study the effect in a more controlled manner, they also measured the elution times and the number of theoretical plates for a neutral (nonadsorbing) analyte in a capillary that had a configuration similar to that used by Herr et al. described earlier, a section of which was coated to create a stepwise change in the wall  $\zeta$ -potential. The elution time was found to increase and the number of theoretical plates was found to decrease sharply with the fraction of the capillary that was coated. The change in bulk flow, as measured by the elution times in these experiments, has recently been shown to be consistent with a simple theoretical model.<sup>16</sup> In addition, a general solution to the problem of EOF through a capillary of arbitrary cross-sectional shape and  $\zeta$ -potential distribution has been presented<sup>17</sup> in the “lubrication approximation” in which the axial variation in the wall’s  $\zeta$ -potential and cross section takes place over a length scale very much longer than the characteristic capillary radius. Analytical results for flow alteration due to variations in wall  $\zeta$ -potentials in case of planar as well as cylindrical geometries have also been reported by Ajdari<sup>18</sup> and Long et al.<sup>19</sup> The problem of a decrease in resolution of electrophoretic separations due to analyte adsorption has been studied using numerical simulation by several authors.<sup>20–23</sup> An analytical treatment is presented by Štědrý et al.<sup>7</sup> in the case of a linear adsorptive flux of analyte at the wall but neglecting the consequent alteration of the wall’s  $\zeta$ -potential.

In this paper, we examine the experimental data of Towns and Regnier<sup>10</sup> on the decrease in the number of theoretical plates for a neutral (nonadsorbing) analyte in a partially coated capillary. It is shown that the Poiseuille flow component due to the induced pressure gradient obtained by Herr et al., together with the result on bulk EOF in capillaries with axially varying  $\zeta$ -potentials shown by Anderson and Idol<sup>14</sup> adequately explains the experimental data through the mechanism of Taylor dispersion. A brief schematic description of the experiment is provided in the next section, followed by a summary of the necessary theoretical results in Section 2. In Section 3, the theory and experiment are compared, and the method for determining some of the unknown physical parameters from the experimental data is explained. Conclusions are presented in Section 4.

## 1. EXPERIMENTAL SECTION

Towns and Regnier’s<sup>10</sup> experimental set up with a partially coated capillary is depicted schematically in Figure 1. It consists of a microcapillary of length  $L = 100$  cm, and internal diameter  $2r_0 = 50$   $\mu\text{m}$ . A segment of the capillary from the inlet to a distance  $l = 15$  cm is coated with PEI-200. A plug of neutral marker (Mesityl Oxide) is introduced at the inlet and allowed to migrate

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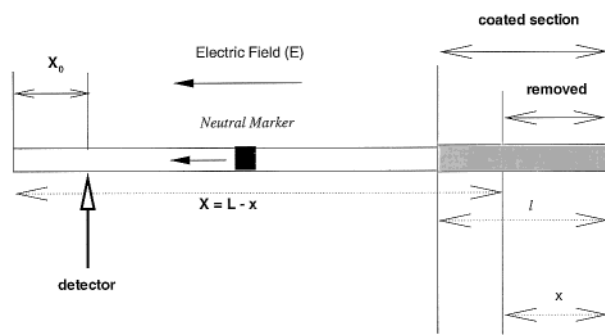


Figure 1. Schematic diagram for the experimental setup of Towns and Regnier for studying EOF and dispersion in a partially coated microcapillary.

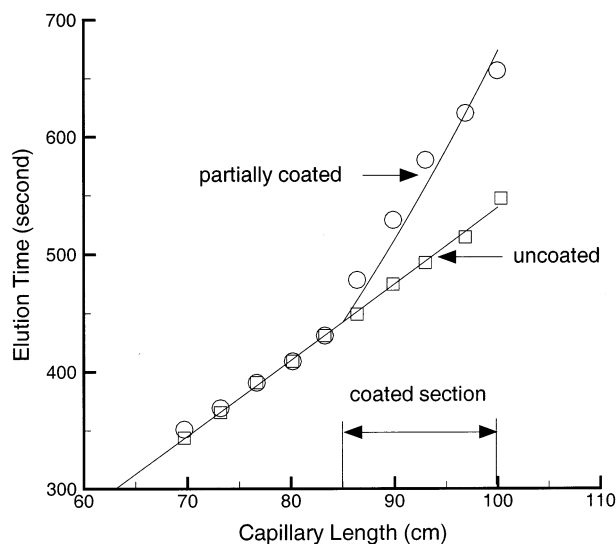


Figure 2. Measured elution time vs capillary length for a partially coated (circle symbols) capillary and an uncoated (square symbols) capillary used as a control. Lines represent the best fit that can be obtained with the theory, eqs 8 and 9 for the partially coated and uncoated cases, respectively.

to the outlet as a result of the EOF. The EOF is generated by applying a potential difference of 30 kV across the capillary, which generates an electric field of  $E = 300$  V/cm directed axially from the inlet toward the outlet. The time of arrival,  $t_e$ , and the axial variation in the concentration averaged over the cross-section  $\bar{c}(x, t_e)$  is measured by a detector placed at a distance  $X_0$  from the outlet. The measurements were repeated by removing a section of length  $x$  from the inlet end of the capillary so that the fraction of the total length of the capillary that was coated,  $p = (l - x)/(L - x)$ , could be varied. Nine such cuts were made, a section of length 3.33 cm being removed at each instance. The applied potential drop was reduced after each cut to keep the electric field strength constant at 300 V/cm. The experimental data consists of the elution time,  $t_e$  for several values of  $X = L - x$  for a coated as well as a similar uncoated capillary, indicated in Figure 2 by the circle and square symbols, respectively. The dispersion in each of these cases expressed as the number of theoretical plates,  $N = X^2/\sigma^2$  was also measured, and these measurements are depicted in Figure 5 by the symbols for the coated (circle) and uncoated (square) capillary.

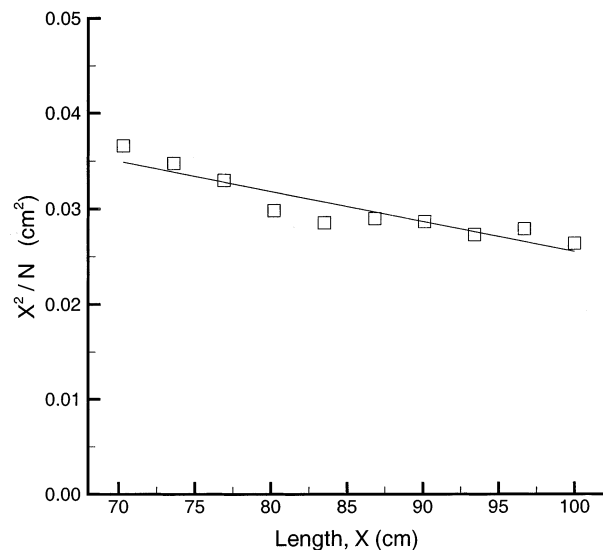


Figure 3. The variance,  $\sigma^2 = X^2/N$  in the case of an uncoated capillary (square symbols), plotted against the capillary length. The line indicates the best linear fit.

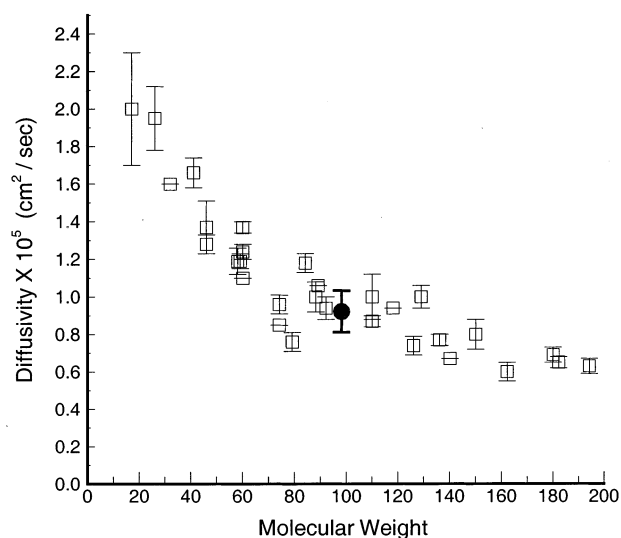


Figure 4. Diffusivities of small organic molecules (square) as a function of their molecular weight, including that of mesityl oxide ( $M_w$  98.1), as computed using the Wilke–Chang formula (filled circle). Vertical bars indicate error estimates (where available).

## 2. THEORY

In the limit of very thin Debye layers, the coupling between the fluidic and the electrical problem is provided by the Helmholtz–Smoluchowski “slip” boundary condition

$$u(r = r_0) = -\frac{\epsilon \zeta}{4\pi\mu} E \quad (1)$$

where  $\epsilon$  is the dielectric constant of the fluid,  $\mu$  is the (dynamic) viscosity, and  $E$  is the applied electric field. In the interior of the fluid, the Navier–Stokes and continuity equations for a constant density fluid apply.

The requirement of mass conservation and incompressibility demands that the velocity along the capillary averaged over the cross-section,  $\bar{u}$ , be independent of the position along the capillary. This bulk flow velocity in the capillary,  $\bar{u}$ , is easily determined

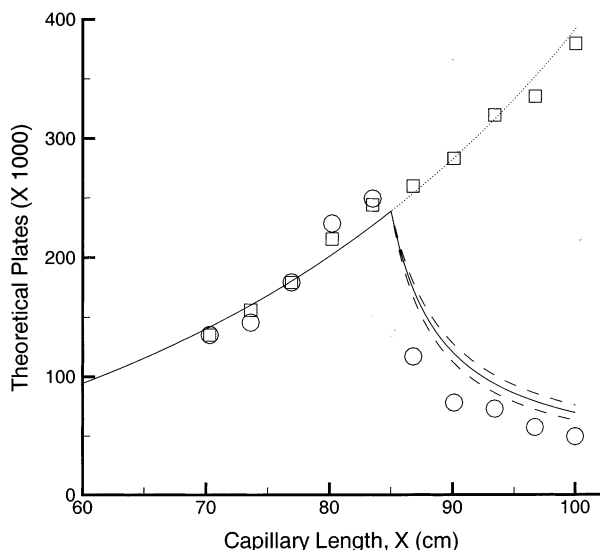


Figure 5. Number of theoretical plates as a function of capillary length as measured experimentally by Towns and Regnier (circle, coated; square, uncoated) and predicted by eq 18 (solid line). Dashed lines on either side correspond to the uncertainty in the value of  $D$  for mesityl oxide. The dotted line is eq 19 for an uncoated capillary.

using the result proved by Anderson and Idol<sup>14</sup> for circular capillaries with  $\zeta$  varying solely in the axial direction.

$$\bar{u} = -\frac{\epsilon\langle\zeta\rangle E}{4\pi\mu} \quad (2)$$

Since

$$\langle\zeta\rangle = \frac{\zeta_0(L-l) + \zeta_1(l-x)}{X} \quad (3)$$

we find that

$$\bar{u} = -\frac{\epsilon E}{4\pi\mu} \frac{\zeta_0(L-l) + \zeta_1(l-x)}{X} \quad (4)$$

where  $\zeta_0$  and  $\zeta_1$  are the  $\zeta$ -potentials of the uncoated and coated sections, respectively. Therefore, for the partially coated capillary, the elution time is

$$t_e = \frac{X - X_0}{\bar{u}} = -\frac{4\pi\mu}{\epsilon E} \frac{X(X - X_0)}{\zeta_0(L-l) + \zeta_1(l-x)} \quad (5)$$

On introducing the notation

$$u_e = -\frac{\epsilon\zeta_0 E}{4\pi\mu} \quad (6)$$

the electroosmotic flow speed in the uncoated capillary and

$$f = 1 - \frac{\zeta_1}{\zeta_0} \quad (7)$$

Table 1

$X_0$ (cm)	$u_e$ (cm/s)	$f$	$D$ (cm <sup>2</sup> /s)	$L$ (cm)	$l$ (cm)	$r_0$ (cm)
16.875	0.154	1.326	$0.938 \times 10^{-5}$	100	15	0.0025

we may express the elution time in the coated capillary in the form

$$t_e = \begin{cases} \frac{X(X - X_0)}{u_e[(l-f)X + f(L-l)]} & \text{if } X > L - l \\ (X - X_0)/u_e & \text{otherwise} \end{cases} \quad (8)$$

for the uncoated case, the elution time is simply given by

$$t_e = \frac{X - X_0}{u_e} \quad (9)$$

In the problem of the partially coated capillary, the geometry is simple enough that the flow profile may be explicitly calculated. The velocity is purely axial and may be written as<sup>15</sup>

$$u_i = u_e \left[ \frac{\zeta_i}{\zeta_0} + 2 \frac{\langle\zeta\rangle - \zeta_i}{\zeta_0} \left( 1 - \frac{r^2}{r_0^2} \right) \right] \quad (10)$$

where  $i = 0$  or  $1$  for the uncoated and coated sections of the capillary, respectively. In eq 10, the first term in the square brackets represents a "pure" electroosmotic flow and the second term is a pressure-driven Poiseuille flow as a result of the induced pressure gradient. Equation 10 implies a discontinuity in the velocity at the boundary of the coated and uncoated sections and assumes a "fully developed" profile at the capillary entrance. In reality, there would be transition regions near the entrance and at the boundary of the coated and uncoated sections. The axial extent of such a transition region may be estimated as  $\Delta X \sim u_e(r_0^2/\nu)$ , since  $r_0^2/\nu$  is the diffusive time scale for radial variations in velocity ( $\nu$  denotes the kinematic viscosity of the fluid). In this problem, the Reynolds number  $Re = (u_e r_0)/\nu \sim 0.1$  (see Table 1) so that  $\Delta X \sim 0.1r_0$ . Because the capillary length is very much larger than this, the neglect of these transition regions in eq 10 is justified.

The variance of the analyte can be calculated from the classical formula

$$\sigma^2 = \sigma_0^2 + 2D_*t \quad (11)$$

where  $\sigma_0^2$  and  $\sigma^2$  are the variance at the initial time and time  $t$ , respectively. Here,  $D_*$  is the effective axial diffusion coefficient given by the sum of the molecular diffusion coefficient,  $D$ , and the Taylor dispersion<sup>12,13</sup> coefficient, so that

$$D_* = D + \frac{r_0^2 u_m^2}{192D} \quad (12)$$

The symbol  $u_m$  represents the maximum magnitude of the parabolic part of the velocity profile in eq 10. Equation 11 assumes that the total advection time  $t_a \sim (X - X_0)/u_e$  is very much larger than the radial diffusion time  $t_d \sim r_0^2/D$  so that the Taylor-Aris dispersion formula, which refers only to the asymptotic limit of large times, may be used. This would be justified if  $t_d/t_a \ll 1$ , or equivalently,  $X - X_0 \gg r_0 Pe$ , where  $Pe = r_0 u_e/D$  is the Peclet number. A simple estimate using the data in Table 1 shows that  $Pe \approx 40$  in this problem so that the condition of validity of the asymptotic limit is  $X - X_0 \gg 40 r_0 \approx 0.1$  cm, a requirement that is certainly satisfied in this experiment.

From equations (10) and (3), we have

$$\frac{u_m}{2u_e} = \begin{cases} -fp & \text{(uncoated section)} \\ f(1-p) & \text{(coated section)} \end{cases} \quad (13)$$

where

$$p = \begin{cases} \frac{X-L+\prime}{X} & \text{if } X > L - \prime \\ 0 & \text{otherwise} \end{cases} \quad (14)$$

is the coated fractional length of the capillary. The travel times through the coated and uncoated sections are, respectively,

$$t_1 = \frac{X-L+\prime}{\bar{u}} = \frac{X}{u_e} \frac{p}{1-pf} \quad (15)$$

$$t_0 = \frac{L-\prime-X_0}{\bar{u}} = \frac{X}{u_e} \frac{1-p-X_0/X}{1-pf} \quad (16)$$

The total dispersion is then

$$\sigma^2 = \sigma_0^2(X) + 2D_*^{(1)} t_1 + 2D_*^{(0)} t_0 \quad (17)$$

where the superscripts 0 and 1 denote the uncoated and coated sections, respectively. We have rewritten  $\sigma_0^2$  as  $\sigma_0^2(X)$  to emphasize the point that the initial variance depends on the injection process and may have a different value for repeated realizations of the experiment with varying lengths of capillaries. On substituting the expressions for  $t_0$ ,  $t_1$ , and  $D_*$  from eqs 12–16 above, we get the following formula for the number of theoretical plates,  $N$ ,

$$N^{-1} = \frac{\sigma^2}{X^2} = \frac{\sigma_0^2(X)}{X^2} + \frac{2D}{u_e X} \frac{1-X_0/X}{1-pf} + \frac{u_e r_0^2 f^2 p(1-p) - p^2(X_0/X)}{24D} \frac{1}{1-pf} \frac{1}{X} \quad (18)$$

where  $p$  is given by eq 14 for the partially coated capillary and  $p = 0$  in the uncoated case.

This expression will be compared to the experimental data of Towns and Regnier.

### 3. COMPARISON OF THEORY WITH EXPERIMENT

Figure 2 allows us to determine some of the physical parameters in the experiment. In the case of an uncoated capillary (square symbols) a linear relation between the elution time and the capillary length,  $t_e = (X - X_0)/u_e$  is apparent from the data. A least-squares fit to the data points gives  $u_e = 0.154$  cm/sec and  $X_0 = 16.875$  cm. The straight line in Figure 2 shows this least-squares fit, together with the square symbols that indicate the measured elution times in an uncoated capillary. The factor  $f$  in eq 8 is chosen so as to minimize the sum of squares of the difference between the predicted and observed elution times in the coated case (round symbols). The best fit is obtained with  $f = 1.326$  and is depicted in Figure 2. Since the PEI-200 coating reverses the charge of the coated section,  $f = 1 - \zeta_1/\zeta_0$  is expected to be larger than unity.

In Figure 3, the variance  $\sigma^2 = X^2/N$  is plotted as a function of  $X$  for each of the data points in the uncoated case. The data are well-represented by the linear fit

$$\frac{X^2}{N} = A + BX \quad (19)$$

where  $A = 0.057012$  cm<sup>2</sup> and  $B = -0.000315$  cm. In the uncoated case, we have, from eq 18,

$$\frac{X^2}{N} = \sigma_0^2(X) + \frac{2D}{u_e} (X - X_0) \quad (20)$$

Therefore,  $\sigma_0^2(X)$  is well-approximated by a linearly decreasing function of  $X$  with a slope whose magnitude is  $>2D/u_e$ . The decrease is due to the fact that the sample is pressure-injected into the capillary by momentarily using a fixed pressure drop,  $\Delta p$ , across it for a fixed interval of time  $\tau$ . Since the fluidic resistance of the capillary is proportional to its length, a shorter plug is injected for a longer capillary. A rough estimate can be given for the magnitude of the effect as follows: The length of sample plug is  $\sigma_0 \sim \bar{u}\tau$ . Even though the flow field during the injection process is not likely to be fully developed, the Poiseuille formula is sufficient for this very rough estimate. Thus,  $\bar{u} \sim (a^2 \Delta p)/(8\mu X)$  so that  $\sigma_0 \sim (a^2 \tau \Delta p)/(8\mu X)$ . Therefore,  $(\Delta \sigma_0^2/\sigma_0^2) \sim -2(\Delta X/X)$ . Since  $\Delta X = 15$  cm and  $X = 100$  cm, the relative change in  $\sigma_0^2$  due to this effect is expected to be about  $-30\%$ . Therefore,  $(\Delta \sigma_0^2/\Delta X) \sim -0.3\sigma_0^2/(\Delta X) \approx 10^{-3}$  cm, whereas  $2D/u_e \sim 10^{-4}$  cm. That is, a decrease in the length of the injected plug with the length of the capillary due to fluidic resistance dominates any increase in variance due to molecular diffusion. In the partially coated case,  $\sigma_0^2$  may be obtained from eqs 19 and 20.

$$\sigma_0^2(X) = A + BX - \frac{2D}{u_e} (X - X_0) \quad (21)$$

To complete the comparison between theory and experiment, it remains to obtain a value of the molecular diffusion coefficient,  $D$ , of mesityl oxide ( $M_w = 98.1$ ). Unfortunately, these data appear not to be available in the literature. However, it may be determined

using one of the empirical formulas for obtaining molecular diffusivities. We will use the Wilke–Chang formula<sup>24</sup> obtained by empirical modifications of the Stokes–Einstein relation,

$$D = \frac{7.4 \times 10^{-8} (\phi M)^{1/2} T}{\mu V^{0.6}} \text{ cm}^2/\text{s} \quad (22)$$

where  $M$  is the molecular weight of the solvent (water),  $\phi$  is an association factor (2.6 is the recommended value if the solvent is water),  $T$  is the temperature in  $K$ ,  $\mu$  is the solvent viscosity in centi-Poise, and  $V$  is the molar volume of the solute at its normal boiling temperature. This last quantity may be determined approximately in units of  $\text{cm}^3/\text{mol}$  by using Schroeder's method: "count the number of C, H, O, and N, add 1 for each double bond, and multiply the result by 7". For mesityl oxide,  $(\text{CH}_3)_2\text{C} = \text{CHCOH}_3$ , this gives  $V = 126 \text{ cm}^3/\text{mol}$ . Therefore, at the experimental temperature of 303.15 K, we have  $D = 0.938 \times 10^{-5} \text{ cm}^2/\text{sec}$ .

Figure 4 compares the calculated value of  $D$  for mesityl oxide with other small organic molecules in aqueous solution. Each square symbol gives the diffusivity and molecular weight of a species in dilute concentration in water at 25 °C. The data are from ref 25. The vertical bars indicate estimates of uncertainty in the data where available. The filled circle indicates the calculated value of the diffusivity of mesityl oxide ( $M_w = 98.1$ ) using the Wilke–Chang formula. The vertical error bar at the level of  $\pm 12\%$  is indicative of the average error in the Wilke–Chang formula ( $\pm 10\%$ ) compounded with the  $\pm 2\%$  uncertainty in Schroeder's method for calculating the molar volume.

Figure 5 shows the number of theoretical plates  $N$ , plotted against the capillary length,  $X$ , using the parameters in Table 1, the expression for the initial variance, eq 21, and eq 18. The solid and dotted lines correspond to the theoretical result for the capillary with and without the PEI-200 coating, respectively. The circles and squares indicate the corresponding experimental data for each of these two cases. The two dashed lines indicate the average uncertainty in the theoretical prediction due to the  $\pm 12\%$  average uncertainty in the value of  $D$ .

The agreement between the predicted and measured values is very good, indicating that flow modification and the consequent Taylor dispersion adequately explain the rapid decrease in  $N$  with increase in the fraction of the capillary that is coated. The fit between theory and experiment can be improved even further if the peak of the curve for the coated capillary is shifted to the left by  $\sim 1$  cm. This is quite plausible, because the boundary between

the coated and uncoated sections is very likely not sharp and an uncertainty of  $\pm 1$  cm is quite probable.

#### 4. CONCLUSION

Adsorption of analytes to capillary walls in CZE is known to be a significant source of band broadening. Such loss of resolution has been observed in experiments by Towns and Regnier,<sup>10</sup> who have raised the possibility that such adsorption could lead to band broadening "by setting up complex flow patterns within the capillary". Such flow modifications have been identified by several authors, who have shown, through theoretical calculations, numerical simulations and experiment<sup>14–18</sup> that adsorption leads to axial nonuniformity in the  $\zeta$ -potential, which induces pressure gradients, which in turn distort the EOF.

The problem of dispersion of a neutral marker in EOF through a partially coated capillary provides a relatively simple problem in which the process of dispersion by flow modification can be studied without the added complexity of the kinetics of the adsorption process. This problem has been investigated by Towns and Regnier,<sup>10</sup> who provide measurements on the decrease in the number of theoretical plates with the coated fraction of a capillary. The effect of the coating on the flow pattern has been independently studied by Herr et al.<sup>15</sup> using a laser fluorescence technique. They show that the flow pattern can be explained by the appearance of an induced pressure gradient due to the axial nonuniformity in the  $\zeta$ -potential.

In this paper, the Taylor dispersion due to the altered EOF was explicitly calculated and shown to be in agreement with the observed decrease in the number of theoretical plates, as reported in the experiment of Towns and Regnier. This work should be considered a necessary first step toward a comprehensive understanding of the more complex and, in a practical sense, the more interesting problem of band broadening of a sample that adsorbs to the wall and therefore creates a space- and time-dependent  $\zeta$ -potential distribution as it travels down the capillary. Such a quantitative theory would be a valuable tool in the hands of designers of "biochips", because it will enable the rapid calculation of performance parameters, such as resolution, peak shape, and stability of elution times, from first principles. Currently, no such predictive capability exists. As a result, expensive and time-consuming trial and error or full scale numerical modeling must be used in the design process.

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