

Effect of Analyte Adsorption on the Electroosmotic Flow in Microfluidic Channels

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The predictability and constancy over time of the electroosmotic flow in microchannels is an important consideration in microfluidic devices. A common cause for alteration of the flow is the adsorption of analytes to channel walls, for example, during capillary electrophoresis of proteins. It is shown that certain experimental data, published by Towns and Regnier (Towns, J. K.; Regnier, F. E. *Anal. Chem.* 1992, 64, 2473–2478.), on the anomalous elution times for proteins in capillary electrophoresis can be explained using a simple model for analyte adsorption that uses a result first reported by Anderson and Idol (Anderson, J. L.; Idol, W. K. *Chem. Eng. Commun.* 1985, 38, 93–106.) on the electroosmotic flux in capillaries with axial variations in ζ -potential. It is suggested that it might be possible to use such a model to dynamically correct for altered elution times in capillary electrophoretic devices.

Alteration of the electroosmotic flow (EOF) due to adsorption of analytes on the walls of microfluidic channels is a serious problem in many bioanalytical systems. Electrophoretic separation of proteins is particularly problematic, because in the pH range commonly used, many proteins are cationic and therefore are readily adsorbed at the negatively charged walls of fused-silica capillaries due to electrostatic attraction. Adsorption also happens, though to a much lesser degree, for anionic proteins as well. Such adsorption alters the wall ζ -potential and, therefore, the EOF through the channel. Electrophoretic separation works by determining the elution time of a specific band of protein over a fixed capillary length. If these elution times are altered in an unknown way due to modification of the EOF, the electrophoretic mobilities of the sample constituents can no longer be determined accurately. Various strategies have been explored to overcome this problem. Polyethyleneimine coatings have been developed that suppress the charged silanol groups on the capillary wall and create a net positive charge on the wall that is stable against changes in pH and repels cationic proteins.¹ Another class of surface treatments attempts to neutralize the wall charge all together. The difficulty with such coatings is that this eliminates or greatly reduces the electroosmotic flow, resulting in greatly increased analysis times in devices relying on a fixed single-point detection method. Nonionic surfactant coatings have been developed² that mask the wall charge due to ionization of the surface silanol groups. Since protein adsorption decreases much more rapidly than any reduction of the EOF, such coated capillaries could be optimized to greatly reduce adsorption without a very large increase in analysis

times. A hybrid approach that has been suggested³ is the use of serially coupled uncoated and coated capillaries. The uncoated capillary generates the EOF throughout the entire column whereas the sample only traverses the coated nonadsorbent section. Such hybrid capillaries are, however, known to create a superposed Poiseuille flow profile^{2,4,5} that reduces the resolution of electrophoretic separations due to Taylor dispersion.

The instantaneous rate of adsorption of analytes to capillary walls is determined by several factors, one being the distribution of analyte concentration in the capillary. This in turn is controlled by the simultaneous effects of diffusion and advection by the fluid flow. The nature and rate of fluid flow is affected by the distribution of ζ -potential on the walls, which changes in a time-dependent fashion due to the adsorption of the analyte. Thus, the problems of the lack of predictability of elution times and zone spreading are a consequence of complex nonlinear physics of which no analytical theory currently exists. Some progress, however, have been made through numerical solution of the governing equations or simplified versions of it, which show some of the qualitative characteristics of the signal seen in actual experiments.^{6–9} An overview of the various causes of poor reproducibility in CZE has been presented recently.¹⁰ Some analytical solutions as well as numerical simulation results on a simplified mathematical model of dispersion have been presented, but these assume a constant electroosmotic flow and neglect the flow modification caused by the alteration of the wall ζ -potential.^{11,12} The impact of this assumption is difficult to judge as no comparison to experiments have been presented.

A systematic experimental study of the effects of protein adsorption on capillary walls was undertaken by Towns and Regnier.¹³ They applied a known voltage drop to a cylindrical capillary of fixed length containing a buffer of known pH. A small amount (10 ng) of protein mixed with a neutral marker (mesityl

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oxide) was introduced as a plug at one end of the capillary. The time of passage of the marker past certain fixed locations along the capillary was noted as the plug was advected along the capillary by the EOF. It is important to note that elution times are being measured for the neutral marker, not for the proteins. Therefore, the experiment is sensitive to the electroosmotic flow, not the electrophoretic migration of the proteins. The experiment was repeated with several proteins ranging in pI from 3.2 to 11.1. The amount of protein recovered at the outlet of the capillary was also measured. It was found that proteins that were cationic at the pH of the experiment were adsorbed the most. Further, both the extent of adsorption and the elution times at the downstream points increased monotonically with the positive charge on the protein. Towns and Regnier noted that even though most of the adsorption took place near the capillary end where the protein was introduced, the largest delay in elution times corresponded to the downstream stations. To further investigate the effect of the changing wall ζ -potential due to protein adsorption, they performed a second set of experiments, where the wall ζ -potential over part of the capillary was altered in a controlled fashion, by giving it a synthetic coat of 20-kDa poly(ethyleneimine), or PEI 200, a polymer that masks the silanol groups on the surface of the fused-silica capillary, giving it a net positive charge. A neutral marker was injected, and its elution time due to the resulting EOF under a fixed applied electric field was recorded for capillaries with varying ratios of the length of treated to untreated sections.

The purpose of this paper is to show that these experiments of Towns and Regnier can be explained using a result first derived by Anderson and Idol.¹⁴ This result is the following; the average EOF velocity over the cross section of a cylindrical capillary whose ζ -potential varies in the axial direction only is given by

$$\bar{u} = \frac{Q}{4\pi r^2} = -\frac{\epsilon \langle \zeta \rangle}{4\pi \mu} E \quad (1)$$

where $\langle \zeta \rangle$ is the average of the ζ -potential over the capillary length, ϵ and μ are the dielectric constant and viscosity of the buffer, E is the applied electric field, Q is the volume flux of fluid, and, r is the capillary radius. That is, the given capillary is "equivalent" as far as the total fluid flux is concerned to a uniform capillary of "effective" ζ -potential $\langle \zeta \rangle$. This result can be extended to channels of arbitrary shapes and arbitrary ζ -potential distributions as long as axial variations in the cross section and the ζ -potential takes place over a length scale that is large compared to a linear dimension characterizing the cross section of the channel.¹⁵ The effective radius and ζ -potentials are then given by certain weighted averages over the channel walls; they are determined completely by the channel geometry and nature of the wall charge distribution. In eq 1 as well as its generalizations,¹⁵ the Debye layer thickness is assumed negligible in comparison to the channel width.

In the next section, flow through a partially coated capillary is considered. Analytical results are first derived for elution times, which are then compared to the experiment of Towns and Regnier. In the following section, their experiment on protein adsorption is considered. Analytical results on elution times are derived and

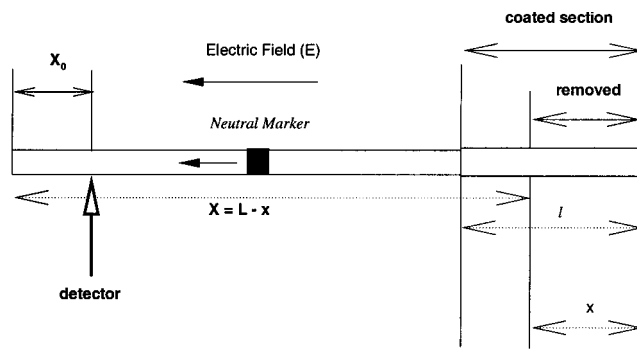


Figure 1. Schematic diagram illustrating the experiment of Towns and Regnier on EOF in a cylindrical capillary with a section coated with PEI 200 to alter its ζ -potential. The capillary is cut in order to remove a section of length x from the inlet end. In the experiment, x is varied by making successive cuts, while the potential drop is simultaneously adjusted in order to keep the electric field constant.

compared with the experimental results, using a simple model for protein adsorption. Conclusions and some possible applications of these results to the design of capillary electrophoretic systems are pointed out in the concluding section.

FLOW THROUGH PARTIALLY COATED CAPILLARIES

The sketch in Figure 1 illustrates schematically the setup for the experiment of Towns and Regnier on partially coated capillaries. EOF is set up in a fused-silica microcapillary (of length L and ζ -potential ζ_0) by applying an electric field (E) along its length. A small plug of a neutral marker (mesityl oxide) is introduced at the inlet, and the time required by the marker to travel to a detector placed at a distance X_0 from the outlet is measured. Next, the measurements are repeated after shortening the capillary, by removing a section of length x from the inlet while reducing the voltage across the capillary at the same time, to keep the electric field constant. Nine successive sections, each of 3.3-cm length were removed, and the elution time was measured in each case to obtain 10 data points with x ranging from 0 to 30 cm. The above experiment is then repeated after replacing the fused-silica capillary with one in which the ζ -potential over a length l from the inlet end has been altered from ζ_0 to ζ_1 by coating with PEI 200. If $x > l$, one expects the elution times to be the same as that obtained with a capillary without the PEI 200 coating, which serves as a "control" to establish that any changes in the elution time is a result of coating with PEI 200 and not due to some extraneous factors. If $x < l$, the elution time is found to be altered, and these correspond to a partially coated capillary.

Theory. The elution time to the detector, $t_0 = (1 - X_0/X)t_c$, where t_c is the elution time across the whole length of the capillary. We will now present a theoretical derivation for t_c based on fluid mechanical principles.

Since the fluid is incompressible, by the continuity equation, the velocity along the capillary averaged over the cross section, \bar{u} , is independent of the position along the capillary, s . Since

$$\zeta(s) = \begin{cases} \zeta_0 & \text{if } s < L - l \\ \zeta_1 & \text{otherwise} \end{cases} \quad (2)$$

$\langle \zeta \rangle$ may be easily evaluated, so that by eq 1 we have

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$$\bar{u}(s, t) = -\frac{\epsilon E}{4\pi\mu} \frac{\zeta_0(L-l) + \zeta_1(l-x)}{L-x} \quad (3)$$

Therefore, for the partially coated capillary, the elution time is

$$t_e = \frac{L-x}{\bar{u}} = -\frac{4\pi\mu}{\epsilon E} \frac{(L-x)^2}{\zeta_0(L-l) + \zeta_1(l-x)} \quad (4)$$

It is assumed here that the neutral marker is simply advected by the bulk velocity of the flow and any spreading of the marker due to molecular or shear-induced diffusion may be neglected as a first approximation. To facilitate comparison with the experimental data, eq 4 may be expressed in terms of $X = L - x$, the section of the capillary remaining after removal of part of the coated section:

$$t_e = \begin{cases} \frac{X^2}{u_e[(1-f)X + f(L-l)]} & \text{if } X > L-l \\ X/u_e & \text{otherwise} \end{cases} \quad (5)$$

where

$$u_e = -(\epsilon\zeta_0 E / 4\pi\mu) \quad (6)$$

is the electroosmotic flow speed in the uncoated capillary and

$$f = 1 - \zeta_1/\zeta_0 \quad (7)$$

Since the PEI 200 coating reverses the charge on the capillary wall, we would expect f to be greater than unity. We will compare the prediction of eq 5 with the experimental results of Towns and Regnier in the next section.

Comparison with Experiment. In Figure 2 the square symbols represent the elution times measured by Towns and Regnier for an uncoated capillary of various lengths. A linear relation between the elution time and the capillary length, $t_0 = (1 - X_0/X)t_e = (X - X_0)/u_e$ is clear, and the least-squares fit to the data points give $u_e = 0.154$ cm/s, and $X_0 = 16.875$ cm. The circular symbols indicate the measured elution times for a partially coated capillary. Towns and Regnier reported the total length of the capillary, $L = 100$ cm, and the length of the coated section, $l = 15$ cm. The factor f in eq 5 is chosen so that the $\sum(t_0^{\text{theory}} - t_0^{\text{observed}})^2$ is minimized. Here t_0^{theory} is the predicted elution time using a specific value of f and t_0^{observed} is the corresponding experimental value reported in ref 13. The summation is performed over the last five data points corresponding to a PEI-200 coated capillary. The best fit is obtained with $f = 1.326$. The theoretical curves for the elution times for coated and uncoated capillaries is shown by the dashed and solid lines, respectively. The fit is seen to be excellent, suggesting that the measurements are consistent with Anderson and Idol's theoretical result on effective ζ potentials. The actual value of the ζ -potential in the coated section, which depends on the pH, the coating thickness, and various details of the chemical treatment has not been provided in ref 13; therefore, the value obtained with the best fit for f cannot be checked against experiment. However, it is known

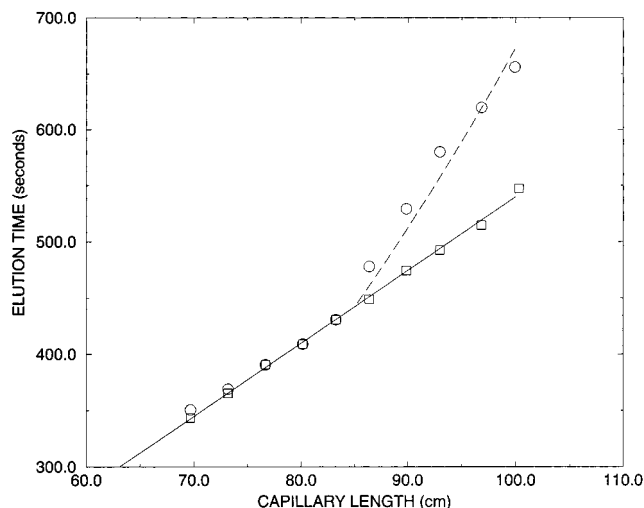


Figure 2. Elution time as a function of capillary length in the experiment of Towns and Regnier using uncoated capillary (squares) and capillary with a section coated (circles). Solid line (uncoated) and dashed line (coated) represent the theoretical results presented in this paper. Data points and symbols correspond to Figure 5 of ref 13.

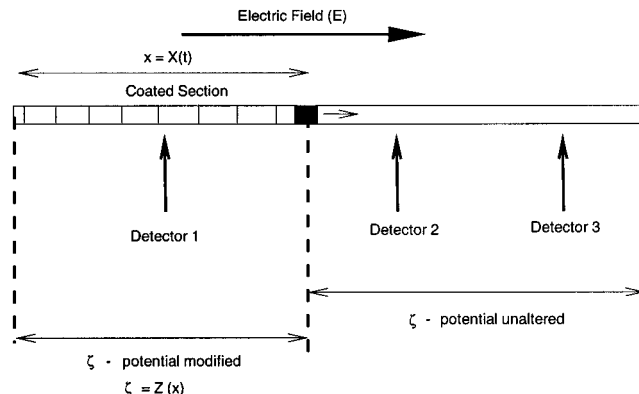


Figure 3. Schematic diagram illustrating the experiment of Towns and Regnier on elution times of a neutral marker mixed with cationic proteins in an uncoated cylindrical capillary. The plug travels from left to right while coating the capillary section that it traverses (shaded) thus altering the ζ -potential behind it. Three detectors are located along the capillary and they measure the arrival times of the plug.

that the PEI 200 coating has the effect of reversing the charge on the capillary, so that $f = 1 - \zeta_1/\zeta_0$ is expected to be larger than unity.

ALTERATION OF EOF DUE TO PROTEIN ADSORPTION

Figure 3 depicts schematically the setup for Towns and Regnier's experiments on the flow modification due to protein adsorption. A small amount of protein mixed with a neutral marker (mesityl oxide) is introduced at the left end and moves from left to right under the influence of the known applied electric field, E . In doing so, part of the protein is adsorbed onto the capillary wall, so that the section of capillary to the left of the injected plug has an altered ζ -potential. In the next section, we derive analytical expressions for elution times, using a simple model for the effect of analyte adsorption on the ζ -potential. In the following section, these are compared to the experimental data of Towns and Regnier.

Theory. Let us, for the moment, ignore here the detailed modeling of the physical process by which protein is transferred from the plug onto the wall. Instead, we assume that as the plug moves along the capillary, it leaves behind a zone of altered ζ -potential whose axial variation is known. Thus, if x denotes distance from the injection end of the capillary and if $x = X(t)$ is the position of the plug at time t , we could represent the ζ -potential at any time as

$$\zeta(x, t) = \begin{cases} Z(x) & \text{if } x < X(t) \\ \zeta_0 & \text{if } x > X(t) \end{cases} \quad (8)$$

where an appropriate model must be chosen for $Z(x)$. The flow in this case is unsteady; however, the time scale of variation is L/u_e , where L is the capillary length and u_e is a characteristic velocity defined by eq 6. The ratio of the unsteady term to the viscous term is the ratio of u_e^2/L to $\nu u_e/a^2$ (ν is the kinematic viscosity and a is the capillary radius) which is $(a/L)R_e$, where $R_e = u_e a/\nu$ is the Reynolds number based on capillary radius. Since both a/L and R_e are very small (Stokes flow), the flow may be regarded as quasisteady. Therefore, we may apply the result (1) of Anderson and Idol, for the flow speed averaged over the cross section. The plug is advected by this bulk flow and it also spreads due to Taylor diffusion induced by the velocity shear caused by the variable ζ -potential.⁴ Since the length of the plug remains very small compared to the capillary length during its passage from the injection point to the detectors, we will treat the plug as essentially a point source that is advected by the bulk flow \bar{u} . Therefore, we have the following differential equation describing the motion of the plug

$$\frac{dX}{dt} = \bar{u} = -\frac{\epsilon \langle \zeta \rangle E}{4\pi\mu} \quad (9)$$

The right-hand side would in general be a function of X . To explicitly evaluate it, we need to know the distribution $Z(x)$. This distribution, however, is determined by the complex nonlinear physics of advection, diffusion, and deposition of the analyte; a full analytical solution of this coupled problem is difficult. A useful simplification is to adopt an ad hoc form for the function $Z(x)$ motivated by physical insight. Such a simplified model is presented next. It should be noted that, except for possible dispersive spreading of the plug, eq 9 is a rigorous result; only the equations after this point are dependent on the ad hoc model for $Z(x)$.

Model for $Z(x)$. According to the observations of Towns and Regnier, for a strongly adsorbing analyte, most of it is adsorbed near the entrance of the capillary. To make this more quantitative, we may consider an exponential model for the distribution of ζ -potential behind the plug:

$$Z(x) = \zeta_0 + (\zeta_1 - \zeta_0) \exp(-\alpha x) \quad (10)$$

where α is a positive parameter of dimension inverse length that determines the strength of the adsorption and therefore, also, the degree of localization of the adsorption region. Strong adsorption corresponds to large values of α ; in this case, most of the alteration in ζ -potential is restricted to the entrance region of the capillary, near $x = 0$. Weak adsorption, on the other hand, corresponds to

small values of α . In this case, the plug coats the capillary almost uniformly, creating an altered ζ -potential behind it, ζ_1 , which is different from the unaltered ζ -potential, ζ_0 ahead of it.

On using eq 10 in eq 8 we get

$$\langle \zeta \rangle = \zeta_0 [1 + (f/p) \{ \exp(-p\xi) - 1 \}] \quad (11)$$

where for convenience we have introduced

$$p = \alpha L \quad (12)$$

$$\xi = X/L \quad (13)$$

$$\tau = (u_e/L)t \quad (14)$$

On substituting the above expression for $\langle \zeta \rangle$ in eq 9 and rewriting in terms of the dimensionless variables, we have the following differential equation for the position of the plug as a function of time:

$$\frac{d\xi}{d\tau} = 1 + \frac{f}{p} [\exp(-p\xi) - 1] \quad (15)$$

This equation may be reduced to a linear one on making the substitution $\eta = \exp(p\xi)$ so that

$$d\eta/d\tau + (f-p)\eta = f \quad (16)$$

which, together with the initial condition $\eta(0) = 1$ (since $\xi(0) = 0$) admits the closed form solution

$$\eta = \frac{1}{f-p} [f - p \exp\{(p-f)\tau\}] \quad (17)$$

The solution may be rewritten in terms of the original dimensional variable X ,

$$\frac{X}{L} = \frac{1}{p} \ln \left[\frac{f - p \exp\{(p-f)u_e t/L\}}{f-p} \right] \quad (18)$$

or, on inverting it, we obtain for the elution time $t = t_e$ corresponding to station $X = X_e$,

$$t_e = \frac{L}{u_e} \frac{1}{p-f} \ln \left[\frac{f}{p} + \left(1 - \frac{f}{p}\right) \exp\left(\frac{pX_e}{L}\right) \right] \quad (19)$$

It is easy to show that, in the limit $p \ll 1$, that is, weak adsorption, eq 19, may be approximated by

$$t_e \approx \frac{L}{fu_e} \ln \left(1 - \frac{fX_e}{L} \right)^{-1} = \frac{X_e}{u_e} + \frac{f}{2u_e L} X_e^2 + \frac{f^2}{3u_e L^2} X_e^3 + \dots \quad (20)$$

where the Taylor expansion in f could be useful if f also happens to be small, that is, if the ζ -potential is only slightly altered by the adsorbing analyte.

Comparison with Experiment. In Figure 4 the symbols show the elution times for seven proteins with pL ranging from 6.2 to

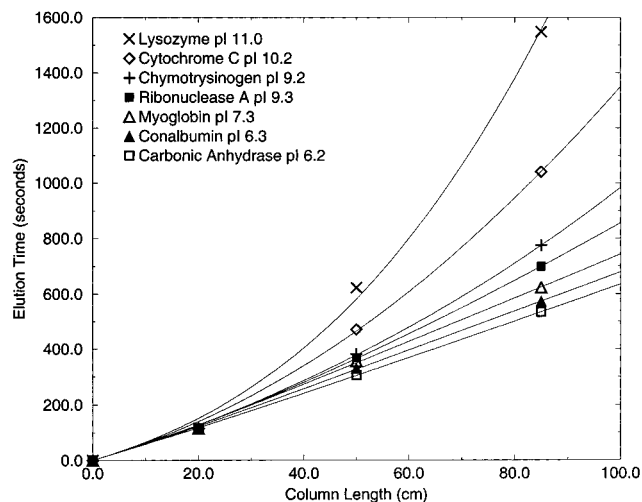


Figure 4. Comparison of Towns and Regnier's experimental data on elution times (symbols) with that calculated from eq 19 (solid line) using the values of the parameters f and p shown in Table 1. The top line corresponds to parameters from the first row in the table, and successive lines correspond to successive rows. The lowest line corresponds to the protein with the weakest affinity for the wall, carbonic anhydrase, which corresponds to $f = 0.285$, indicating only a slight alteration of the wall ζ -potential. Symbols and data are from Figure 4 of ref 13.

Table 1

protein	pI	f	p
lysozyme	11.0	2.745	3.000
cytochrome <i>c</i>	10.2	2.028	2.541
ribonuclease A	9.3	1.119	1.350
chymotrypsinogen	9.2	1.134	2.151
myoglobin	7.3	1.347	4.720
conalbumin	6.3	0.900	4.500
carbonic anhydrase	6.2	0.285	1.250

11.0 at stations that are 20, 50, and 85 cm from the injection point. The experimental data points are taken from Figure 4 of ref 13. The remaining four proteins with pI below 6.2 reported in ref 13 essentially follow the linear relation $t_e = X/u_e$, indicating no adsorption or alteration of the EOF. These are not shown in Figure 4. The first data point, which corresponds to detector 1 in ref 13 shows negligible effect of protein adsorption. Using the elution time, $t_e = 114.3$ s to detector 1 (located at $X_e = 20$ cm), we obtain $u_e = X_e/t_e = 0.175$ cm/s.

Since the values of the ζ -potentials or the distribution of proteins in the capillary are not known, the parameters f and p are obtained by requiring that the theoretical elution times predicted by eq 19 provide the best possible fit to the experimentally measured times. This was achieved by finding the minimum of $S(f, p) = \sum (t_e^{\text{theory}} - t_e^{\text{observed}})^2$ in a search window $f_{\text{max}} > f > f_{\text{min}}$, $p_{\text{max}} > p > p_{\text{min}}$ with some plausible choice of f_{min} , f_{max} , p_{min} , and p_{max} using a simple FORTRAN routine. Here t_e^{theory} is the predicted elution time using a specific value of f and p and t_e^{observed} is the corresponding experimental value reported by Towns and Regnier. The summation is performed over detectors 2 and 3. Table 1 shows the "best values" determined in this way for each of the seven different proteins. In Figure 4, the lines correspond to the elution times calculated from eq 19 using the values of f and p listed in Table 1 as a function of the column length X_e . The

seven lines (from top to bottom) in the figure correspond to the entries in the seven successive rows of Table 1 for f and p . The fit to the data is seen to be very good. The good fit was, however, obtained using an ad hoc functional form for $Z(x)$ and adjustable parameters p and f ; therefore, agreement between theory and experiment cannot be claimed on this basis. Nevertheless, the weaker claim may be made that the simple model presented here is at least consistent with the experimental data of Towns and Regnier.

CONCLUSIONS

In relation to the experiment on elution times with various proteins, Towns and Regnier observed that, "It is surprising, however, that the least impact on transport time was in the first section of the capillary, where protein adsorption is greatest. Transport velocity of the neutral marker was reduced most in the section of capillary where no protein was adsorbed." In this paper, we have attempted to provide a clear explanation of this and other aspects of these experiments based on fluid mechanical principles. The explanation of this apparently surprising behavior is that, due to the requirements of the continuity equation for an incompressible fluid, the bulk velocity at every cross section of the capillary at a given time is the same. Further, this bulk velocity is determined by Anderson and Idol's formula and is proportional to the axially averaged ζ -potential. As the latter quantity decreases monotonically in time as more and more protein gets adsorbed to the wall (irrespective of *where* it is adsorbed), the flow velocity in the entire capillary slows down, accounting for elution times being larger for stations located furthest from the injection point. This is the essential physical content of eq 19.

Second, the data provided by Towns and Regnier on partially coated capillaries have been used here to verify the analytical results first reported by Anderson and Idol on the bulk EOF in cylindrical capillaries. The experimental data are seen to be consistent with the theory.

Finally, eq 19 suggests a possible method for correcting for the lack of reproducibility of elution times in CZE. This may be achieved simply by adding at least two interrogation windows between the injection point and the final detector and including in the sample either a neutral marker or a species of known electrophoretic velocity. The arrival times of this marker species at the two intermediate windows may then be used to "calibrate" the instrument dynamically at run time by using the data in eq 19 to determine the two constants f and p . Multipoint interrogation would allow an even more accurate calibration of the constants f and p . Since the arrival time at the detector identifies the species, the lack of reproducibility of elution times is a serious problem, and various methods have been proposed ranging from continuous monitoring of the bulk flow velocity (see ref 16 and references therein) to suppression of EOF by using various synthetic coatings on the capillary walls. The advantage of the proposed method is that it does not require major modifications to the hardware; instead, it is based primarily on a new way of processing the output signal.

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