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Evaluation of fundamental properties of a silica capillary used for capillary electrophoresis

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ABSTRACT

A model was developed that accounts for the decrease in the electroosmotic flow in a capillary electrophoresis system when the buffer concentration is increased. Important parameters are: the initial charge per unit area at the silica capillary wall, Q_o; a compact layer of molecules of constant thickness d_o that exists between the capillary wall and the buffer; and the equilibrium constant, K_{wall}, between the cations in the buffer and adsorption sites on the silica capillary. An excellent fit of an equation derived from the model to experimental results was obtained. Values for the above parameters were determined in a number of different buffers and the effect of pH, buffer composition and column coatings on these parameters was evaluated.

INTRODUCTION

Capillary electrophoresis (CE) has a wide range of applications in the separation of charged species. Among the advantageous features of this technique are rapid analysis times and high resolution. Migration times and resolution are governed by the applied voltage, the electrophoretic mobilities (μ_{ep}) of the samples being separated and the electroosmotic flow (μ_{eo}). A reduction in the electroosmotic flow can lead to an improvement in the resolution of components in a sample. However, control of electroosmosis is not straightforward; modifications of the chemical environment of the column are necessary.

There have been several approaches to the control of the electroosmotic flow with particular emphasis on its reduction or elimination. The use of coated capillary columns [1–5] has been employed to reduce greatly the electroosmotic mobility, with the additional benefit of minimizing the adsorption of proteins to the silica capillary wall. The addition of organic modifiers such as methanol or acetonitrile also leads to a reduction in μ_{eo} [6–10], as does an increase in the concentration of the buffer [3,10–15]. The pH of the buffer also has an effect on the electroosmotic mobility [12,13,16–18]. Recently the application of a radial electric field has been shown to increase or decrease the electroosmotic flow [19]. With the exception of the latter instance, all of the above techniques involve altering the chemical properties of the buffer or the capillary wall to reduce the zeta potential, which is proportional to the electroosmotic flow.

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The zeta potential is the potential induced by the negatively charged capillary wall acting on a layer of positive ions next to it. The zeta potential is proportional to the product of the number of charges on the silica surface and the thickness of the counter ion layer. These in turn are affected by the nature of the ions in the counter ion layer, the pH of the buffer and the equilibrium between the cations in the buffer and the silica surface. An exact relationship between the zeta potential and the fundamental properties of the system has not been derived and therefore it has not been possible to obtain values for the thickness of the counter ion layer or the number of charges per unit area at the silica surface. Such values would be useful in the evaluation of various methods used in the reduction of the electroosmotic flow. One method may be more effective than another simply because it controls one key parameter. On the other hand, different methods may have the same effect on the properties of the silica surface, so that there is no advantage of one method over another.

In order to gain some insight into the fundamental properties of the chemistry at the capillary wall, we have developed an equation relating the electroosmotic mobility to the concentration of the buffer. From a fit of the equation to experimental results, it is possible to obtain values of the initial charge at the capillary surface and the equilibrium constant between the silanol groups on the silica surface and the cations in the buffer. The model incorporates a mass-action (Langmuir isotherm) law for neutralization of the wall, and a compact layer of mobile cations, buffer and water molecules in close proximity to the capillary surface. The actual structure of the compact layer is open to speculation. The effect of various strategies used to control the electroosmotic flow such as changing the pH, the addition of organic modifiers and column coatings are discussed in terms of changes induced in the fundamental properties of the silica column.

EXPERIMENTAL

Instrumentation

The research CE instrument used was similar to those described previously [1,20]. A 50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) 1 m long with a window at 50 cm was suspended between two buffer reservoirs. The applied voltage was supplied by a 30-kV Glassman (Whitehouse Station, NJ, USA) high-voltage power supply. Voltages were adjusted to keep the power below 0.06 W. A 20% solution of acetone was used as a neutral marker. The detector was a modified Jasco UV detector set to 265 nm. Injections were done by raising the sample vial 3 in. above the end reservoir and then dipping the capillary column into the sample vial for 10 s.

The column was etched for 30–60 min with either 0.1 *M* NaOH, KOH, LiOH or RbOH at the start of each day. Distilled water was used to rinse out the column before introducing the buffer. For the phosphate buffer systems, the column was rinsed with 0.015 *M* phosphoric acid before introducing the buffer. When the buffer concentration was changed, the column was re-etched for 5–15 min before introducing the new buffer. The 2-(N-morpholino)ethanesulfonic acid (MES)–histidine (His) buffer was the one exception in that the column was not etched before the introduction of a different concentration to avoid contamination with NaOH. At least two measurements of the electroosmotic flow were conducted for any given buffer.

Chemicals

Several organic and inorganic buffers were used. In the pH 9.55 regime, 3-(cyclohexylamino)-2-hydroxy-1-propanesulphonic acid (CAPSO) was adjusted to the desired pH with NaOH. When methanol was used in the CAPSO–NaOH buffer, the pH was adjusted after the methanol had been added. The CAPSO–NaOH–CH_3OH buffers contained 8 wt.% of methanol. In the pH 6.05 regime, MES was adjusted to the required pH with either NaOH, KOH, LiOH, RbOH or histidine. A phosphate buffer, made from KH_2PO_4 and phosphoric acid, was used at pH 3.8. The organic acids and histidine were purchased from Sigma (St. Louis, MO, USA), RbOH and LiOH from Aldrich (Milwaukee, WI, USA) and methanol, NaOH, KOH, KH_2PO_4 and phosphoric acid from J. T. Baker (Phillipsburg, NJ, USA).

Control of heating

The passage of current through a resistive buffer will cause heating of the buffer in proportion to the applied voltage and the current. If enough heat is generated the buffer viscosity will decrease, which leads to an increase in the measured electroosmotic mobility. Therefore, it is important to work in a regime where very little heat is being generated or to use some method of cooling the column. We chose the former approach.

In Fig. 1, the electroosmotic mobility is plotted as a function of power (applied

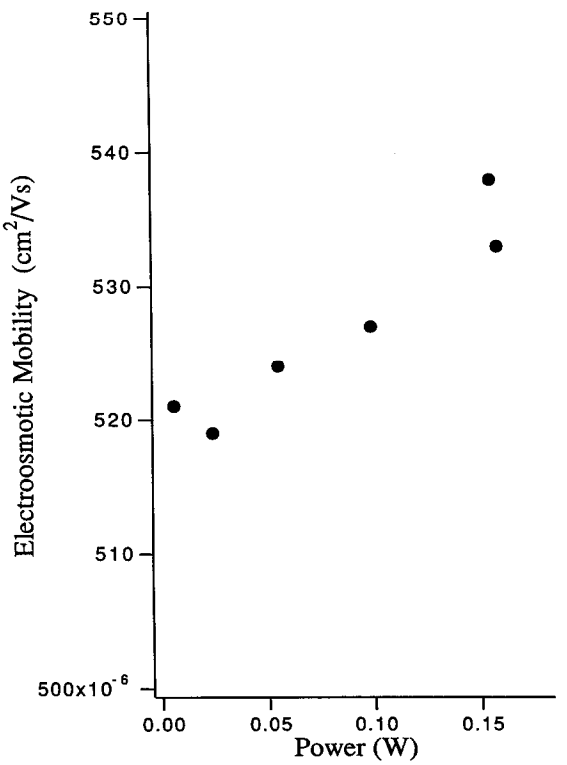


Fig. 1. Measured electroosmotic mobility as a function of power. Buffer is 20 m*M* MES–8 m*M* NaOH (pH 6.05). The applied voltage ranged from 5 to 25 kV.

voltage × measured current) for a 20 m*M* MES–8 m*M* NaOH (pH 6.05) buffer. Above 0.06 W, μ_{eo} begins to increase with increasing power. We limited the studies to powers less than 0.06 W and the applied voltage was adjusted accordingly. At this power, the internal temperature of the column was below 26°C [21].

DERIVATION OF THE ELECTROOSMOTIC FLOW MODEL

We are interested in the formulation of an equation for the electroosmotic mobility in terms of easily measured quantities such as the buffer concentration and the buffer viscosity. In a simple system, where there is no coating on the inside of the column and there are no surfactants in the buffer, the negatively charged capillary wall is balanced by a layer of positive ions in the vicinity of the capillary surface. Some of the positive ions may be adsorbed onto the capillary surface, but the other cations remain in the buffer and are relatively mobile. A potential is induced by the negatively charged capillary surface acting on the positive ions; the magnitude of the potential diminishes as one moves further away from the capillary wall. When an external electric field is applied parallel to the wall, the positive ions in the buffer move toward the negative electrode, thereby setting up the electroosmotic flow. The electroosmotic mobility is proportional to the potential at the interface between the immobile capillary wall (including any adsorbed ions) and the freely moving buffer. This potential is called the zeta potential (ζ). An exact expression for μ_{eo} is [22]

$$\mu_{eo} = \frac{\varepsilon\, \varepsilon_0\, \zeta}{\eta} \tag{1}$$

where η is the buffer viscosity, ε is the dielectric constant of the buffer and ε_0 is the permittivity of free space. The potential induced by the capillary surface is compensated by positive ions over an effective distance x (perpendicular to the wall), such that the electroosmotic mobility can be rewritten as

$$\mu_{eo} = \frac{Q\, x}{\eta} \tag{2}$$

where Q is the charge per unit area at the interface between the capillary wall and the buffer solution and x is usually referred to as the thickness of the counter-ion layer.

As a first approximation to obtaining a relationship between μ_{eo} and the buffer concentration, we assume that Q is a constant (at a given pH) and that x is dependent on the buffer concentration. If the potential at the capillary surface decreases exponentionally as one moves away from the capillary surface, we can apply the Gouy–Chapman model to our system. In such a case the counter-ion layer thickness would be equal to δ, the Debye–Hückel thickness [11]; the distance δ corresponds to a drop in potential equivalent to a value that is 1/e of the potential at the capillary surface. The Debye–Hückel thickness is inversely proportional to the square root of the ion concentration, $[M^+]$ [23]. For a system in which both the buffer and counter-ion are monovalent the following expression can be derived from eqn. 2:

$$\mu_{eo} = \frac{Q}{\eta\, K' \sqrt{[M^+]}} \tag{3}$$

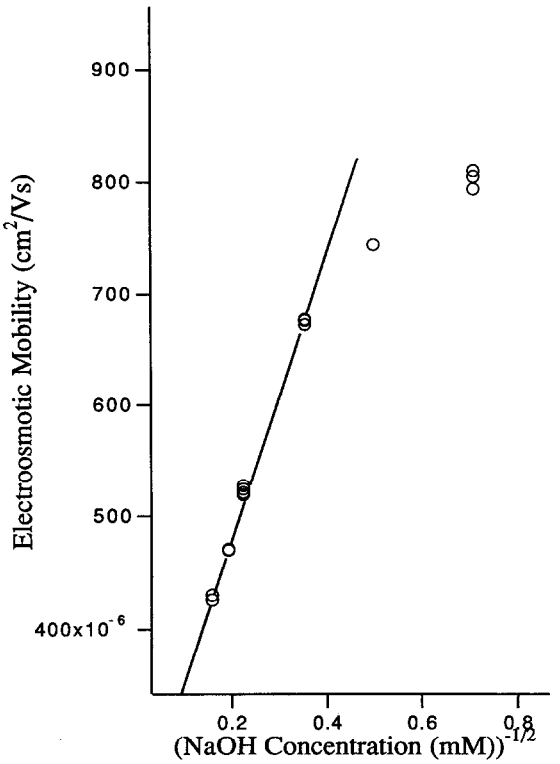


Fig. 2. Measured electroosmotic mobility plotted against the reciprocal of the square root of the NaOH concentration. The line drawn through the points at higher concentrations is there to emphasize the linearity in this regime. Buffer as in Fig. 1; voltages adjusted to keep the power below 0.06 W.

where K' is equal to $3.2 \cdot 10^9$ m^{-1} (mol l^{-1})$^{-1/2}$ for a dilute, aqueous system at 25°C [23]. Although eqn. 3 accounts for the observed decreases in μ_{eo} with increasing buffer concentration at a given pH, it is not exact. As seen in Fig. 2, a plot of μ_{eo} *vs.* [NaOH]$^{-1/2}$ has a non-zero intercept. This offset can be remedied by postulating that the double-layer thickness is the sum of a compact layer of fixed thickness, d_0, and the Debye–Hückel thickness, δ; this modification is analogous to the Stern modification of the Gouy–Chapman model:

$$x = d_0 + \delta = d_0 + \frac{1}{K' \sqrt{[M^+]}} \tag{4}$$

As x is the thickness of a mobile charge layer defined by eqn. 2, the compact layer of thickness d_0 and the diffuse layer thickness, δ, in the above equation are also considered to be mobile. While the above correction takes into account a non-zero intercept when μ_{eo} is plotted against [NaOH]$^{1/2}$, the non-linearity of the plot at low buffer concentrations is still a problem.

It may be argued that the non-linearity in the plot is due to a breakdown in the

assumption that the charge per unit area at the interface (Q) is constant. It seems likely that Q is also a function of buffer concentration; as the buffer concentration is increased, more cations are adsorbed on the capillary wall and the number of exposed silanol groups is proportionately reduced. Cations are known to adsorb to silica surfaces and can be held there by forces in addition to electrostatic attraction; however, the situation is not well understood [24]. We examined a simple adsorption mechanism [15] as shown below:

$$M^+ + SiO^-(H_2O) \rightarrow SiO^-M^+ + H_2O \tag{5}$$

where SiO^- is a silanol group on the surface, M^+ is a monovalent cation other than H^+ and SiO^-M^+ is a silanol group with an adsorbed cation. The equilibrium constant based on this mechanism can be written as follows:

$$K_{wall} = \frac{[SiO^-M^+]}{[M^+][SiO^-]} \tag{6}$$

The charge per unit area at the interface (Q) is simply the concentration of SiO^-. We denote the total number of ionized silanol groups at the capillary surface as Q_0:

$$Q_0 = [SiO^-] + [SiO^-M^+] \tag{7}$$

The following expression for Q can then be derived from eqns. 6 and 7:

$$Q = \frac{Q_0}{1 + K_{wall}[M^+]} \tag{8}$$

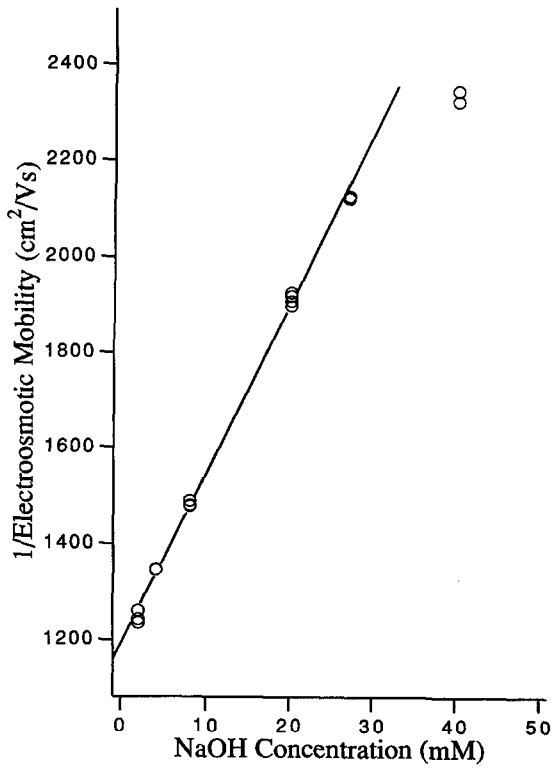
According to eqn. 8, $1/Q$ is proportional to $(1 + K_{wall}[M^+])$. If one assumes that the thickness of the counter-ion layer (x) is constant, then based on eqn. 2, $1/\mu_{eo}$ is also proportional to $(1 + K_{wall}[M^+])$. A plot of $1/\mu_{eo}$ *vs.* [NaOH] is shown in Fig. 3. Linear behavior is observed at lower concentrations; however, there is slight curvature at higher concentrations, indicating that the simple adsorption model does not completely describe the concentration dependence of μ_{eo}.

A more accurate description of the chemistry involved at the surface of the capillary column may have both Q and x being dependent on the buffer concentration. Q is affected by the adsorption of cations on the capillary surface and x is affected by the number of cations in the buffer. A schematic diagram is shown in Fig. 4. By substituting eqn. 4 for x and eqn. 8 for Q in eqn. 2, the following expression for the electroosmotic mobility as a function of buffer concentration can be obtained:

$$\mu_{eo} = \frac{Q_0}{\eta\,(1 + K_{wall}[M^+])}\left(d_0 + \frac{1}{K'\sqrt{[M^+]}}\right) \tag{9}$$

Eqn. 9 provides an excellent fit to our data on the dependence of μ_{eo} on cation concentration; a few examples are shown in Fig. 5. χ^2 values are of the order of 10^{-10}.

Parameters determined from a fit of the above equation to the MES–NaOH data



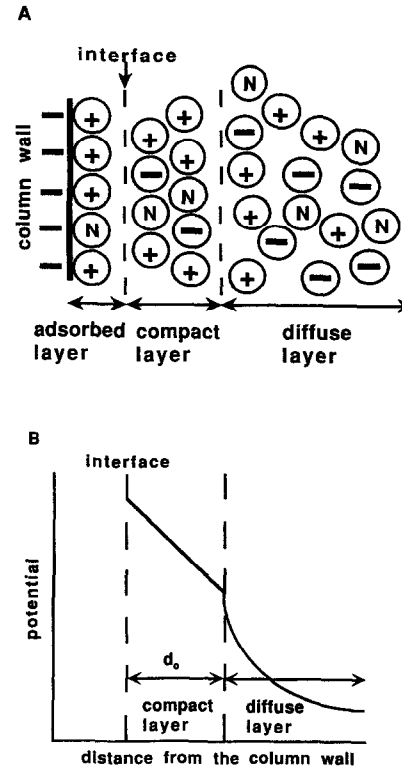


Fig. 3. Reciprocal of the measured electroosmotic flow plotted against NaOH concentration. Conditions as in Fig. 2. The line drawn through the points at lower concentration is used to emphasize the linearity of the data in this regime.

Fig. 4. (A) Representation of our model of the parameters associated with the silica capillary. (B) Potential of the system as a function of the distance away from the wall.

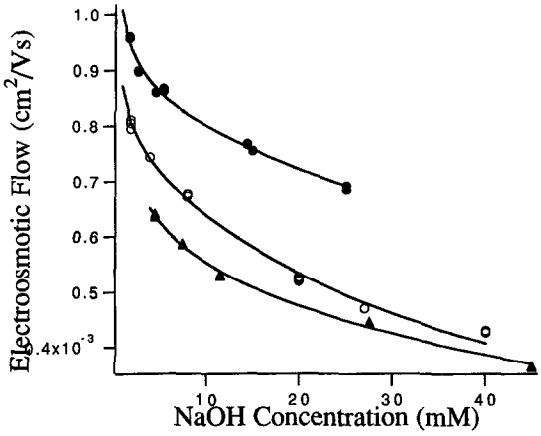


Fig. 5. Electroosmotic flow *vs.* NaOH concentration for three different buffers. ● = CAPSO–NaOH (pH 9.55); ○ = MES–NaOH (pH 6.05); ▲ = CAPSO–NaOH–CH_3OH (pH 9.55). The lines drawn through the points in each data set are the result of a fit of the data to eqn. 9.

TABLE I

CONSTANTS DERIVED FROM A FIT OF EQN. 9 TO THE DATA

System		K_{wall} (l mmol^{-1})	d_0 ($\times 10^{-8}$ m)	Q_0 ($\times 10^{16}$ sites m^{-2})
pH	Components			
9.55	CAPSO–NaOH	0.0093	3.9	1.3
	CAPSO–NaOH–CH_3OH	0.011	1.3	2.9
6.05	MES–LiOH	0.017	1.6	1.4
	MES–NaOH	0.020	3.9	1.1
	MES–KOH	0.034	3.2	0.9
	MES–RbOH	0.023	2.8	1.4
	MES–His	0.034	0.95	2.2
3.8	KH_2PO_4	0.084	2.8	0.50
	KH_2PO_4, coated column[a]	0.083	0.88	1.1

[a] Ref. 3.

are shown in Table I. The compact layer thickness (d_0) was determined to be $3.9 \cdot 10^{-8}$ m. This distance is much larger than the diameter of a hydrated sodium cation, which is of the order of $3 \cdot 10^{-10}$ m [25]. Hence, it appears that the compact layer is more than a single layer of cations. A better description involves several layers of ordered hydrated cations interspersed among buffer molecules and buffer anions. The total number of ionized silanol groups per unit area on the capillary wall is *ca.* 0.3% of the surface SiOH groups, assuming that there are between $4 \cdot 10^{18}$ and $5 \cdot 10^{18}$ SiOH groups per square meter [24]. Because of the good fit to the data, we feel that eqn. 9 will be useful for the elucidation of key parameters relating to the chemistry at the silica capillary surface.

APPLICATIONS OF THE MODEL

In this section we utilize eqn. 9 to evaluate Q_0, d_0 and K_{wall} in a number of different buffer systems by measuring μ_{eo} at several different concentrations of a given buffer. We were interested in elucidating the changes in the above parameters caused by variations in the type of cation present in the buffer, the buffer pH, the addition of organic modifiers to the buffer and the applications of column coatings.

Effect of buffer cation

The choice of the counter-ion used in a given buffer can have a large effect on the electroosmotic flow, as shown in Table II. At a given concentration and pH, μ_{eo} is greatest for MES–NaOH, while decreasing slightly for MES–RbOH. The electroosmotic flows in MES–KOH, MES–LiOH and MES–His were all roughly the same and about 30% less than that for MES–NaOH. The variation in μ_{eo} with buffer cation cannot be accounted for by changes in the hydrated cation radius, which increases from Rb to K to Na to Li (Table II). For all five buffer systems, we measured μ_{eo} at five different concentrations of the buffer and then fitted eqn. 9 to the results to obtain the parameters associated with the capillary wall. Values of Q_0, d_0 and K_{wall} are included in Table I.

TABLE II

PHYSICAL CONSTANTS AND MEASURED ELECTROOSMOTIC FLOW FOR MES BUFFERS WITH DIFFERENT COUNTER-IONS

Cation	Li	Na	K	Rb	His
Conductivity[a] ($cm^2\ \Omega^{-1}\ mol^{-1}$)	38.69	50.11	73.50	–	–
Ion mobility[b] ($\times 10^{-4}\ cm^2\ V^{-1}\ s^{-1}$)	4.01	5.19	7.62	7.92	–
Hydrated cation radius[c] (Å)	2.1	1.6	1.1	1.1	–
Current[d] (μA)	0.78	0.70	1.18	1.6	0.61
Electroosmotic flow[d,e] ($\times 10^{-4}\ cm^2\ V^{-1}\ s^{-1}$)	5.03	8.0	4.98	7.43	5.55

[a] Ref. 23.
[b] Ref. 25.
[c] Calculated from the ion mobility; see ref. 25, p. 827.
[d] Measured at 25 kV in a buffer of 5 m*M* MES and 2 m*M* cation (5 m*M* histidine).
[e] Uncertainties are less than 1% based on day-to-day reproducibility.

The variation in the electroosmotic flow with the type of buffer cation is a complicated combination of factors; however, some trends are apparent. Low values of the compact layer thickness predominate over changes in the other parameters, leading to the observed low values of μ_{eo} for MES–LiOH and MES–His. An anomaly is the MES–KOH system, which has a d_0 value similar to those for the MES–NaOH and MES–RbOH systems but has a much lower electroosmotic flow. Here a greater value of the equilibrium constant appears to be important.

One striking result is that the value of Q_0 is roughly the same for the alkali metal cations in the MES buffers, indicating that the initial number of SiO^- groups does not depend on the type of cation used. However, in the case of the MES–His buffer, the value of Q_0 is twice that of the other MES buffers. It appears that the bulky histidine may act to mediate the charge repulsion between SiO^- groups on the surface and allow for more SiO^- groups to be present in a given area.

Effect of pH

To study how changing the pH affects the condition of the capillary column, we measured μ_{eo} at several concentrations of a CAPSO–NaOH buffer at pH 9.55 and at several concentrations of a potassium phosphate buffer at pH 3.8. Eqn. 9 provided an excellent fit to the data (Fig. 5) and values of Q_0, d_0 and K_{wall} are included in Table I.

A comparison of CAPSO–NaOH values with those for MES–NaOH at pH 6.05 shows that both Q_0 and d_0 were unchanged. It is surprising to find that the initial charge at the capillary wall changed so slightly from pH 6 to 9. From our analysis, the increase in the electroosmotic flow from $7.4 \cdot 10^{-4}\ cm^2\ V^{-1}\ s^{-1}$ at pH 6.05 (4 m*M* NaOH) to $8.7 \cdot 10^{-4}\ cm^2\ V^{-1}\ s^{-1}$ at pH 9.55 (4 m*M* NaOH) is attributable to the reduction in K_{wall} by a factor of two. In our simple adsorption model, pH effects on K_{wall} were not included. However, it is known [26] that equilibrium constants are dependent on the concentration of other ions in the medium. The origin appears to be due to electrostatic attraction between ions in solution for the ions involved in the equilibrium process of interest. In our case, at higher pH values, the equilibrium shifts in the direction of more free cations; the increased OH^- concentration is apparently removing adsorbed cations from the capillary surface.

In the lower pH regime, there are significant changes in Q_0. Q_0 increases from 0.50 in a pH 3.8 KH_2PO_4–H_3PO_4 buffer to 0.9 in a pH 6.05 MES–KOH buffer. The equilibrium constant (K_{wall}) decreases from pH 3.8 to 6.05 as it did in going from pH 6.05 to 9.55; the mechanism may be the same, namely the increased solvation of the cation (K^+ in this case) by the increased OH^- concentration. Finally, the compact layer thickness (d_0) does not appear to be influenced by changes in pH, provided that the type of cation remains the same.

Effect of methanol

The addition of methanol to a buffer is known to lead to a reduction in the electroosmotic mobility [6–10], but the exact mechanism remains unclear. We are able to obtain values of Q_0, d_0 and K_{wall} in a pH 9.55 CAPSO–NaOH–MeOH buffer which are included in Table I. As can be seen, the addition of methanol leaves the equilibrium constant unchanged. However, Q_0 more than doubled; it appears that methanol shields charged sites from each other, thus allowing more to exist in a given area. The same increase in Q_0 was observed in the MES–His buffer at pH 6.05 where histidine is capable of the same kind of shielding. The addition of methanol also leads to a shrinking of the compact layer, which may be the result of improved solvation of the charges in this region. The reduction in the compact layer thickness overrides the increase in Q_0 to result in an overall reduction in the electroosmotic velocity (Fig. 5).

Effect of column coating

Bruin *et al.* [3] used coated capillary columns to improve the separation of proteins in a phosphate buffer. In addition to the elimination of protein adsorption to the capillary wall, a reduction in the electroosmotic mobility was noted. Because μ_{eo} was measured at several concentrations, we were able to fit eqn. 9 to their results and obtain values for the fundamental parameters, which are included in Table I. In addition, we measured the electroosmotic mobility in an uncoated column at several concentrations using the same phosphate buffer. The results of a fit of eqn. 9 to our data are also included in Table I.

A comparison of values for the coated and uncoated columns shows that the application of a coating to the capillary wall had no effect on the equilibrium constant, indicating that the coating does not alter the chemistry between the silica wall and the cations in the buffer. However, the use of a coating reduced the thickness of the compact layer by a factor of three; the origin of this effect is not clear. An increase in Q_0 is observed with the coated column, presumably owing to better shielding of the SiO^- groups by the coating. As seen with the addition of methanol to the buffer, the reduction in the thickness of the compact layer overwhelms the increase in Q_0 to lead to an overall reduction in μ_{eo}.

Summary of individual influences

From the above analyses of various column treatments, several trends are evident. The equilibrium constant between the buffer cations and adsorption sites on the capillary wall is insensitive to column coatings and to the presence of organic modifiers in the buffer. The equilibrium constant decreases as the pH is increased and is sensitive to the type of cation used in the buffer. The effect of adding methanol to the buffer is similar to that of coating the column, namely leading to an increase in Q_0 due

to better shielding of surface SiO^- groups from each other. In both instances the increase in Q_0 is overshadowed by a decrease in d_0 which leads to the observed reduction in the electroosmotic flow. The use of histidine in the MES–His buffer has the same effect as the addition of methanol or the coating of the column. Increasing the pH causes an increase in Q_0 with the greatest effect occurring between pH 3.8 and 6.05, which is in agreement with some recent studies of the electroosmotic flow as a function of pH [17]. The thickness of the compact layer (d_0) is not affected by changes in pH provided that the type of cation in the buffer is kept constant.

CONCLUSIONS

We have developed a model of electroosmotic flow as a function of buffer concentration that is a useful tool for analyzing the surface parameters of a fused-silica capillary column under a variety of conditions. An excellent fit of the model to our own data and to the results of other work was obtained. From our analysis, it appears that both the addition of organic modifiers and the coating of the capillary column lead to a reduction in the electroosmotic flow by the same mechanism, namely the reduction in compact layer thickness which overwhelms the increase in initial charge at the surface. Raising the pH leads to an increase in Q_0 at lower pH, but above pH 6.05 Q_0 increases only slightly. The equilibrium constant between buffer cations and the silica surface decreases as the pH is increased. The type of cation used in the buffer can also lead to a reduction in μ_{eo} through a combination of factors.

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