

Effects of Buffer pH on Electroosmotic Flow Control by an Applied Radial Voltage for Capillary Zone Electrophoresis

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Electroosmotic flow has been shown to be controlled via an applied radial voltage. Many factors determine the effectiveness of this control, and one major factor is buffer pH. In this study the effectiveness of the applied radial voltage for controlling electroosmotic flow while varying buffer pH is examined. Previously developed theory is applied and compared to experimental results for a pH range from 1.4 to 6.32. Analysis time is dramatically reduced by applying a radial voltage for separation of a peptide mixture at pH 1.4. Theory predicts laminar flow profiles under some conditions when applying this technique. However, experimental evidence at pH 6.32 and 1.4 shows no evidence of band broadening from a laminar flow profile. Theoretical and experimental results indicate the largest range of effective electroosmotic flow control via an applied radial voltage occurs at low pH. Furthermore, a sigmoidal relationship between electroosmotic flow and applied radial voltage is clearly apparent under these conditions. In contrast, at high buffer pH (>6) the relationship appears to be linear and is only over a limited range of flow velocities.

INTRODUCTION

Capillary electrophoresis (CE) is a high-efficiency, low-volume separation technique. This and related techniques (micellar electrokinetic capillary chromatography (MECC), capillary gel electrophoresis, etc.) separate charged and neutral species in aqueous solution with a wide range of applicability.^{1,2} Applications include cations, anions, and neutrals from single atomic ions³ to proteins and nucleic acids with molecular weight in excess of 130 000 Da⁴. One important factor in the applicability of CE to such a large range of analytes is electroosmotic flow (EOF). This flow, generated at the wall of the capillary, typically causes the elution of cations, anions, and neutrals at one end of the capillary, allowing the use of a single detector.⁵ In addition, a term for electroosmotic flow appears in the equations for both resolution and efficiency in MECC⁶ and CE.⁵ Hence, the control of EOF may have a large effect in improving resolution and efficiency and is one of the most important aspects of controlling reproducibility in CE.

Electroosmotic flow may be altered in many ways. These include derivatizing the inner surface of the capillary,⁷⁻⁹

altering the buffer pH¹⁰⁻¹² or concentration,^{10,13-15} the addition of surface active species (surfactants, glycerol, etc.)^{7,8,16} or organic modifiers¹⁷ to the buffer, and the application of applied radial voltage to the capillary wall.¹⁸⁻²²

One of these methods, the variation of pH, has a strong effect on electroosmotic flow.^{10,11,23} The variation of buffer pH may essentially stop electroosmotic flow at low values (pH <2.5) and induce a maximum flow at high values (pH >7) in fused silica capillaries. Thus, buffer pH defines the full range of electroosmotic flow with the value of the electroosmotic flow coefficient ranging from approximately zero to 1×10^{-3} cm/V s.¹¹ Since a term appears for electroosmotic flow in the resolution equation for CE and MECC, buffer pH may be optimized for resolution. However, many separations, especially biological, are limited in pH by the instability of biomolecules or by adsorption of the solutes to the capillary wall.¹² Furthermore, pH alters the charge on proteins and peptides and therefore may alter their electrophoretic mobilities.²⁴⁻²⁶ By altering their mobilities the separation of protein and peptide mixtures may be realized by optimized pH. Thus, pH may be adjusted to optimize analysis time, resolution, and efficiency or be held at a given value. In situations where the pH range that can be employed is limited, it would be advantageous to vary the electroosmotic flow independently.

Independent control of EOF with an applied radial voltage has been investigated in several recent publications.¹⁸⁻²² This work has identified some important factors which influence the ability of the radial voltage to control EOF and to define the limits of this effect. Theoretical aspects of this effect come from established double-layer theory from electrochemistry and colloid science.^{20,27,28} Experimentally, we have developed a novel means to create this effect with a Nafion-

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coated CE capillary,²⁰ and this apparatus has many practical advantages to previous systems which control EOF by applied radial voltage. The new design system leaves the polyimide coating intact on the capillary and simply covers it with a flexible ionomer. Hence, the capillaries retain their flexibility and strength in these experiments.

Although the control of EOF appears to have several advantages as outlined above, under specific conditions this technique might lead to laminar flow and associated band broadening.²² In all published experimental apparatuses there are sections of the capillary which are not covered by the radial voltage control sheath. Since the effect is assumed to be directly radial inward, then the flow at the inner wall of the capillary can be expected to differ from the unsheathed sections versus the sheathed sections. This may lead to laminar flow and band broadening. A series of experiments are presented to determine the magnitude of this effect.

In this paper, the effect of buffer pH on the control of electroosmotic flow by applied radial voltage is examined, both theoretically and experimentally. For a buffer pH ranging from 1.4 to 6.32 excellent agreement is found between theory and experimental data for the rate of EOF. In addition, an application of this method to the separation of peptides at low pH is examined. Finally, the effect of EOF control on band broadening is examined.

THEORY

Theoretical considerations for the control of EOF with an applied radial voltage have been developed.^{19,20} Briefly, these considerations are that the ionized surface silanol groups of the fused silica capillary indirectly define the ζ potential and, hence, EOF. These ionized groups generate surface charge density, σ_{Si} , which is related to solution pH by the following relationship:

$$\sigma_{Si} = \gamma / (1 + ([H^+]/K)) \quad (1)$$

where γ is the total of both the ionized and protonated surface silanol group concentrations, $[H^+]$ is the bulk buffer hydrogen ion concentration, and K is the dissociation constant. This surface charge density is related to the ζ potential by the following relationship:

$$\zeta = \exp(-\kappa x) (2kT/e) \sinh^{-1} (\sigma_{Si} [500\pi/\epsilon_b RCT]^{1/2}) \quad (2)$$

and

$$\kappa = 2\eta_o Z^2 e^2 / \epsilon_b kT \quad (3)$$

where ϵ_b is the permittivity of the buffer, η_o is the viscosity of the buffer, Z is the electronic charge, e is the elementary charge, T is the temperature, κ is the inverse Debye length, x is the thickness of the counterion, k is the Boltzmann constant, C is the electrolyte concentration, and R is the molar gas constant. The ζ potential is related to electroosmotic flow velocity, ν_{eo} , by

$$\nu_{eo} = \zeta (\epsilon_b / \eta_o) E_{app} \quad (4)$$

where E_{app} is the separation potential field strength. By applying eqs 1, 2, and 4, it can be seen that buffer pH directly effects EOF through the ionization of surface silanol groups. This effects the EOF through variation of the ζ potential according to eq 2 which predicts the characteristic sigmoidal shape of the relationship between buffer pH and EOF shown by previous studies.^{10,11,23}

Surface charge may also be induced via a radially applied voltage. The relationship between applied radial voltage, V_r ,

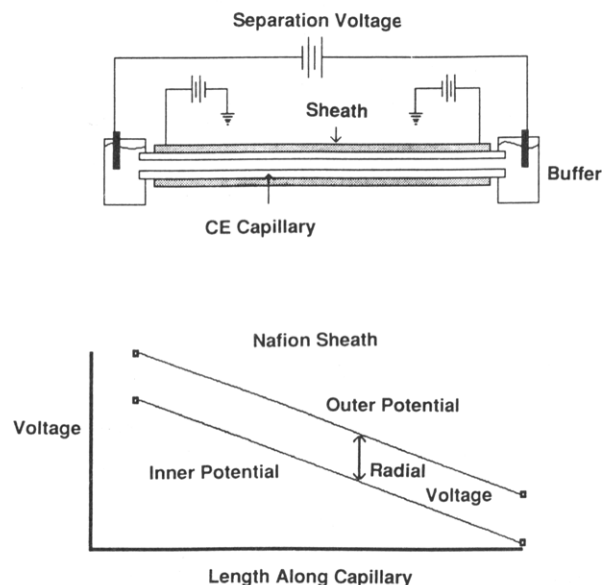


Figure 1. Schematic of the experimental apparatus used to control EOF by applied radial voltage. The high-voltage lead in the buffer on the left is isolated in an interlock Plexiglas box. The sheath (Nafion coating) and both high-voltage leads are isolated in a separate interlock box. The bottom section shows the expected potential drop inside the capillary assuming a homogeneous buffer versus length along the capillary. Also plotted is the potential field of the Nafion outer coating which is manipulated by the independent adjustment of each power supply, defining the end point potential. In this apparatus, the applied radial voltage (inner potential–outer potential) may be manipulated to be constant across the length of the capillary.

and surface charge density, σ_v (due only to effects of V_r), has been described^{19,20} as

$$\sigma_v = (\epsilon_Q V_r / R_I) (1 / \ln (R_O / R_I)) \quad (5)$$

where ϵ_Q is the permittivity of the fused silica capillary, R_O is the outer radius of the capillary, and R_I is the inner radius of the capillary.

This surface charge density due to the applied radial voltage enhances or depletes the surface charge density due to the ionization of the surface silanol groups (σ_{Si}) and therefore directly effects ν_{eo} . The nature of the interaction between σ_{Si} and σ_v is assumed to be additive. By inserting both sources of surface charge into eq 2 and combining with eq 3, a relationship between applied voltage, buffer pH, and EOF may be developed as follows:

$$\nu_{eo} = (\epsilon_b / \eta_o) E_{app} \exp(-\kappa x) (2kT/e) \times \sinh^{-1} ((\sigma_v + \sigma_{Si}) [500\pi/\epsilon_b RCT]^{1/2}) \quad (6)$$

EXPERIMENTAL SECTION

Apparatus. The Nafion-sheathed capillary for application of an applied radial voltage was described in a previous study.²⁰ Briefly, the system used two Plexiglas interlock boxes designed for operator safety. The high-voltage lead for the separation potential and the injection end of the polyimide-coated fused silica capillary were enclosed in the first box. The second box contained the Nafion-coated section of the capillary and high-voltage electrodes on either end of the sheath to adjust the radial potential field (Figure 1). The radial voltage field was manipulated by calculating the linear voltage drop in the buffer-filled interior and adjusting the outer sheath voltage to match the gradient, offset at the experimental value (Figure 1, bottom). The capillaries used for the variation of buffer pH studies were 50 cm in length, 10- μ m i.d.; 141- μ m o.d. and 25- μ m i.d.; 250- μ m o.d. (Polymicro, Phoenix, AZ). Capillaries were used as received without further modification. Electroosmotic flow was monitored with phenol as the neutral marker. Detection was carried out on-column through a window where the polyimide was removed

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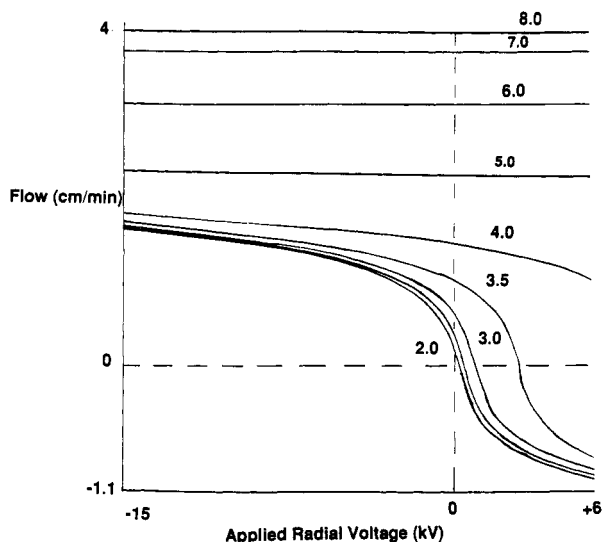


Figure 2. This graph shows a plot of eq 6 for a series of buffer pH values. The specific values for all variable input parameters are separation potential, 140 V/cm; capillary, 50 cm \times 10- μ m i.d. \times 150- μ m o.d.; temperature, 298 K; concentration, 1 mM; dissociation constant, 1×10^{-7} ; width of counterion, 1.5×10^{-10} m; dielectric coefficient, 78; viscosity, 0.000 89 kg/m s; and surface silanol group concentration, 5×10^{14} groups/cm².

by heat. Detection was by absorbance (Linear 200, Reno, NE) at 214 nm.

This apparatus was used to separate a series of peptides. A 25 mM phosphate buffer at pH 1.4 was used with a 20- μ m-i.d., 144- μ m-o.d., and 52-cm-long capillary. In one set of experiments, the capillary was coated with a thin layer of highly conductive silver paint (Ernest F. Fullham, Latham, NY; P/N 14810) and attached to a 0–30 kV power supply (Spellman, Plainview, NY).

Chemicals. Solutions were made from NaH₂PO₄ (Sigma, St. Louis, MO) and adjusted to the desired pH with H₃PO₄ (Baker Chemical, Phillipsburg, NJ) or NaOH. Phenol solutions were made from "phenol liquefied" (Fisher, Fairlawn, NJ). Peptide standards were prepared in operating buffer. Nafion (a perfluorosulfonic acid polymer) was obtained as a 5% solution in methanol/water from Solution Technology, Inc. (Mendenhall, PA). All chemicals were used as received with no further purification. Twice distilled (Megapure-Corning, Corning, NY) water was used for all buffers and solutions.

RESULTS AND DISCUSSION

Effect of pH on EOF Control. In previous publications, applied radial voltage has been shown to alter EOF to a large degree.^{18,22} This dynamic method of EOF control shows great promise, and the investigation of the fundamental properties of this process is important. The controlling effect is based on a capacitive charge across the wall of the capillary resulting from the application of the radial voltage. This capacitive charge is assumed to affect the ζ potential in an additive manner with those charges that are due to ionization of surface silanol groups. Since the surface charge is a function of the buffer pH, the effectiveness of control by radial voltage should also be affected by pH. The effect of varying the pH has been theoretically examined and the resulting plot is shown in Figure 2. This figure is a plot showing the theoretical flow versus applied radial voltage (eq 6), where pH values are set from 2 to 8. The specific input values are listed in the figure legend. As the hydrogen ion concentration increases, the operand decreases and lowers EOF due to the reduced number of surface charges from the silanol ionization. This, in turn, allows a greater relative control with the applied radial voltage. The less charge on the surface due to ionized functional groups on the inner surface allows greater absolute control of the flow by the applied radial voltage. Also, as control of EOF

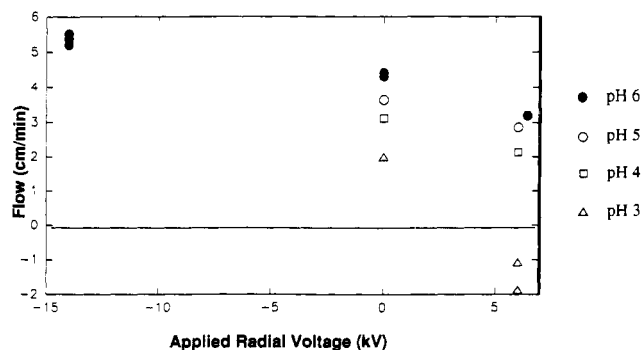


Figure 3. Graph of experimental results for control of EOF by an applied radial voltage at a series of buffer pH values using phenol as a neutral marker. Data for pH 6 shows two data points for each radial voltage setting. Data for pH 4 and 5 have only one data point per radial voltage setting. The data for pH 3 indicate a reversal of flow for the +6-kV radial voltage setting. Experimental conditions: separation potential, 140 V/cm; capillary, 50 cm \times 25- μ m i.d. \times 250- μ m o.d.; concentration, 1 mM phosphate buffer; detection, absorbance at 214 nm.

increases at lower pH the sigmoidal shape of the hyperbolic inverse sine function becomes apparent.

Electroosmotic flow control by applied radial voltage has also been examined experimentally over the range of pH from 3.00 to 6.00. The results obtained at applied radial voltages of -14 kV, no applied radial voltage, and at +6 kV are shown in Figure 3. Experimental trends in the data are clearly in agreement with the theoretical plots of eq 6 shown in Figure 2. A relatively linear relationship exists between EOF and applied radial voltage for the pH values of 4, 5, and 6 (correlation coefficients of 0.9997, 0.9992, and 0.947, respectively). Experimental data at these pH values (Figure 3), indicates that the EOF is altered over the entire range of applied voltages by 59, 48, and 22%, respectively. The linear relationship between EOF and radial voltage under these experimental conditions has led to the suggestion that a linear relationship exists at all accessible applied radial voltages.^{19,22} However, at lower pH values it appears that EOF velocity approaches an asymptotic limit when controlled by increasing applied radial voltages. It also appears that altering the surface charge by extreme pH values also causes flow to approach this limit, and it is likely to be due to some saturation of the surface/buffer interface. In addition to these interesting effects, the data set obtained at pH 3 shows a large effect on the flow, shows a flow reversal, and is clearly not a linear relationship.

At pH 3.00, EOF can be varied from ca. 3.5 cm/min to ca. -1.87 cm/min at applied radial voltages from -14 to +6 kV, respectively. Figure 4 shows several experimental data points plotted along with a line representing eq 6 (multiplied by 3.9 to match the absolute magnitude of the flow) for these same experimental conditions. At this pH, it is clear that the control of EOF by an applied radial voltage is much more effective in the presence of fewer ionized silanol surface charges than are present at higher pH. It is also clear that eq 6 predicts the sigmoidal shape of this data set. Equation 6 also predicts more effective control of EOF with the 10- μ m-i.d., 140- μ m-o.d. capillary versus the 25- μ m-i.d., 250- μ m-o.d. capillary, although comparing Figures 3 and 4 shows better control for the 25- μ m-i.d. capillary. These relatively small differences have been attributed to capillary to capillary variation; hence, group data sets are considered here. However, the relationship between EOF control and capillary radius is the subject of current study in our laboratory. Electroosmotic flow control does not follow a linear relationship under these conditions, and it would seem that accuracy near the steepest portion of the curve would be difficult. However, since this section of

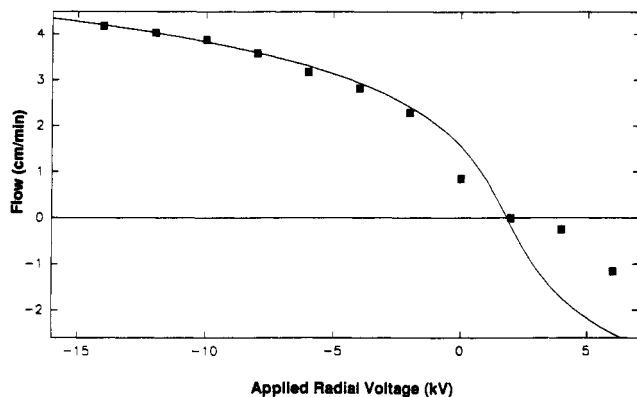


Figure 4. Comparison of experimental data and theory from eq 6 at pH 3.00. Each box represents the average of several neutral marker experiments, with $n = 1$ for data at -14 and 0 kV and $n = 3$ or more for all others. The solid line is theoretical data from eq 6 employing the same variables as in the experiments (multiplied by 3.9 to match the magnitude—see text). Experimental conditions: separation potential, 140 V/cm; capillary, 50 cm \times 10 - μ m i.d. \times 144 - μ m o.d.; concentration, 1 mM phosphate buffer; detection, 214 nm. Additional variables for eq 6: dielectric coefficient, 78 ; viscosity, 0.00089 kg/m²s; counterion diameter, 1.5×10^{-10} m; dissociation constant, 5×10^{-7} ; surface silanol group concentration, 5×10^{14} groups/cm².

the curve occurs near zero flow rate, it becomes experimentally unimportant for many applications.

Values of EOF generated by eq 6 had to be increased by a factor of 3.9 to match the magnitude of the experimental data points in Figure 4. While the trends predicted by the theory are clearly supported by the experimental evidence, the exact magnitude is not. This points out the limitations of the fundamental theory, some of which have been discussed previously.²⁰ The theory developed assumes that a monovalent buffer system and a perfectly cylindrical inner capillary wall are utilized. Also, the dissociation constant for surface silanol groups has been reported at values ranging over 8 orders of magnitude.²⁹ The inherent limitation of the double-layer theory which is based on Debye-Hückel approximations assumes constant viscosity and dielectric properties across the double layer, of which there is much evidence to the contrary.²⁸ Finally, it is not clear what error the unsheathed portions at the ends of the capillary are contributing to the flow. Understanding these assumptions and limitations, the theory, as developed, predicts all of the experimentally observed trends in this study. The usefulness of eq 6 in assessing the effect of pH on EOF control is clearly apparent in Figure 4. In addition, in the absence of any applied radial voltage, eq 6 is consistent with experimental results for varying pH,^{10,11,23} temperature,³⁰ and concentration¹³ in normal unsheathed CE systems.

Enhanced Time of Analysis for Low pH Separations of Peptides. A separation was carried out to demonstrate the utility of EOF control. McCormick¹² has used a low pH buffer system to separate a series of di- and tripeptides while preventing adsorption of these solutes to the capillary wall. At a pH of 1.5, EOF is nearly eliminated and the interaction of the analytes with the wall of the capillary is minimized. In the previous work, the absence of EOF has been described as an advantage due to irreproducibility of this flow from run to run. In the experimental scheme presented in this paper, EOF control reduces the analysis time and the flow is considerably more reproducible owing to independent control. Two electropherograms are shown in Figure 5 for the separation of several peptides. Figure 5a shows the separation in the absence of an applied radial voltage and Figure 5b with

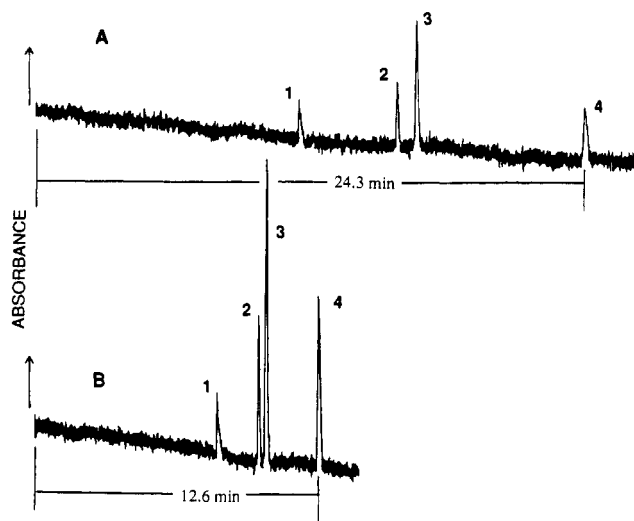


Figure 5. Separation of di- and tripeptides at pH 1.4 with and without EOF control: (A) no applied radial voltage and (B) the same separation with the applied radial voltage of -14 kV. Analytes: (1) L-lysyl-L-lysine, (2) L-glycyl-L-alanyl-L-alanine, (3) L-histidyl-L-phenylalanine, and (4) L-lysyl-L-phenylalanine. Experimental conditions: separation potential, 140 V/cm; capillary, 52 cm \times 20 - μ m i.d. \times 144 - μ m o.d., 44 cm to detector; concentration, 25 mM phosphate buffer; detection, absorbance at 200 nm.

an applied radial voltage of -14 kV. The total analysis time has been nearly divided in half under conditions of EOF control. In addition, the peak height (signal to noise) has improved and all components are completely resolved in the electropherogram with the -14 -kV applied radial voltage.

Use of a Conductive Sheath for EOF Control. Since we realize a possible disadvantage of the EOF control technique is band broadening due to possible laminar flow, this has been investigated further. Theory to date assumes that capacitive charging of the double layer inside the capillary only occurs in the portions of the capillary covered by the sheath. According to the theory previously developed, there should be a large differential in the ζ potential (and therefore flow at the wall) in the unsheathed sections at the ends of the capillary. This effect may lead to a systematic error in experiments where the flow is reversed. In addition, the differential flows might be expected to lead to significant band broadening due to laminar flow profiles near and in these regions. The magnitude of this effect is not great enough to observe it in the separation of the peptides shown in Figure 5. So, to test this predicted band broadening under more extreme conditions, a conductive sheath²⁰ has been applied over the length of the capillary and held at 0 V. Since the radial voltage drop across the fused silica wall over the length of the capillary would be varying in direct relation to the linear potential drop inside the capillary, this should induce differential flow at the wall over the length of the capillary. This is in contrast to the Nafion-coated apparatus where the applied radial voltage is constant everywhere except over the uncoated portions of the capillary. The experiments have involved two probes and two different pH values. Efficiency has been evaluated with phenol (pH 6.32) and L-histidyl-L-phenylalanine (pH 1.4). Theoretical plates (N) have been calculated in the usual manner where $N = 5.54 (t_r/w_h)^2$, where t_r is the elution time and w_h is the peak width at half height. The experimental results are shown in Table I. No evidence of band broadening is present at either pH. In fact, the applied radial voltage appears to slightly increase efficiency. This phenomenon is the subject of further investigation in our laboratory. These initial experiments appear to indicate that the application of this technique does not detract from the advantages of the plug-type flow in electroosmosis.

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Table I. Change in Theoretical Plates upon Application of Grounded Conducting Sheath

experimental condition	theoretical plates ^a	n
pH 1.4 (L-Histidyl-L-phenalanine)		
no sheath	44 327 ± 217	5
conductive sheath (60% of capillary)	59 291 ± 2	5
pH 6.32 (Phenol)		
no sheath	87 700 ± 9200	6
conductive sheath (60% of capillary)	101 000 ± 6300	6

^a Errors are standard deviations.

CONCLUSION

The effects of varying pH on the control of EOF, both theoretically and experimentally, have been examined. A theory has been presented that successfully predicts the range and relationship between applied radial voltage and EOF. The effect of applied radial voltage on EOF is significantly

amplified at low pH relative to high pH. The usefulness of EOF control has also been demonstrated by providing an improvement in the separation of peptides at low pH. Finally, preliminary data to examine the effect of different EOF values along the capillary appears to indicate that, at least for the conditions of EOF control by an applied radial voltage, solute band broadening is not affected.

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