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Direct Control of Electroosmosis and Retention Window in Micellar Electrokinetic Capillary Chromatography

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The ζ potential and the electroosmotic flow in micellar electrokinetic capillary chromatography (MEKC) can be directly controlled by using a radial electric potential gradient across the capillary wall. In this study, the separations of neutral phenylthiohydantoin amino acids as the model system in MEKC with direct control of electroosmosis and retention window are presented. Significant enhancement in the separation resolution of phenylthiohydantoin amino acids under the direct control of electroosmosis is demonstrated.

INTRODUCTION

Many biochemical and pharmaceutical separations involve neutral compounds or mixtures of neutral and charged compounds. Micellar electrokinetic capillary chromatography (MEKC) was therefore introduced by Terabe in 1984[1] as one of the separation modes of capillary electrophoresis specifically for neutral compounds. MEKC involves the addition of surfactant to the aqueous phase at concentrations above their critical micelle concentration. At these concentrations, surfactant monomers tend to form roughly spherical aggregates, or micelles. The neutral compounds are therefore separated on the basis of their differential partitioning between the aqueous phase and the hydrophobic interior of the micelles.

Retention in MEKC is generally based on hydrophobicity with more hydrophobic solutes interacting more strongly with the micellar phase. The retention time of a solute (t_R) that interacts with the micelles will fall in a "retention window" between the elution time (t_o) of a solute that has little or no interaction with the micelle and the elution time (t_{mc}) of a solute that is 100% solubilized by the micelles. One key obstacle in the application of MEKC to the separation of complex sample is its limited retention window. The peak capacity, which is defined as the maximum number of resolved peaks, is clearly dependent on the specific range of retention time. Any measures taken to increase the range of retention window should therefore increase the number of components which can be resolved by MEKC.

The net flow velocity of the micelles can be affected by changing their electrophoretic velocity and/or the electroosmotic velocity. Balchunas and Sepaniak's attempt at increasing the micellar electrophoretic velocity involved the use of a relatively short chain-length surfactant, sodium decyl sulfate (STS), with the aim of forming smaller micelles.[2] Although elution ranges obtained when STS was used were larger than those obtained for sodium dodecyl sulfate (SDS), solute retention time reproducibility was poor with the STS system. Another limitation to this approach is that as surfactant chain length decreases, critical micelle concentration increases dramatically. The high surfactant concentrations needed for micelle formation result in high electric currents, which can cause heat dissipation problems and a degradation of separation efficiency.

A second approach for manipulating the range of retention window involves the adjustment of electroosmotic flow rather than the electrophoretic velocities of the micelles. Reported attempts for affecting the ζ potential and the electroosmotic flow include the use of surface-active agents, buffer pH, buffer composition, temperature, or chemical derivatization of the surface.[3-5] In addition, we recently proposed and demonstrated the use of a radial electric potential gradient across the capillary wall for directly controlling the ζ potential and the electroosmotic flow in capillary zone electrophoresis (CZE).[6-9] Significant improvements in the separation efficiency and resolution of peptide and protein mixtures in CZE were demonstrated with the direct control of electroosmosis.[9] Theoretical aspects of electroosmotic control upon the application of a radial electric potential gradient were discussed by using the capacitor theory[7] and a site-dissociation model[10] at the capillary/solution interface.

As discussed previously,[7] such electronic adjustment of electroosmosis became diminished at high solution pH's such as pH 7. In this study, the direct control of electroosmosis in MEKC is thus investigated at 10 mM buffer solution at pH 3.6. The effect of electroosmotic control on retention window and separation resolution of neutral phenylthiohydantoin amino acids (PTH-amino acids) as the model system is presented and discussed.

EXPERIMENTAL SECTION

The experimental setup in an external grounding configuration for applying a radial electric potential gradient across the capillary wall has been described previously by Holloway and his coworkers.[11] In contrast to the coaxial configuration,[6-9] only one capillary was required in this external grounding setup. As shown in Figure 1, about 70% of the external surface of the silica capillary (with polyimide coating) was coated with a conductive medium such as silver by vacuum deposition. A 1-mm UV window near

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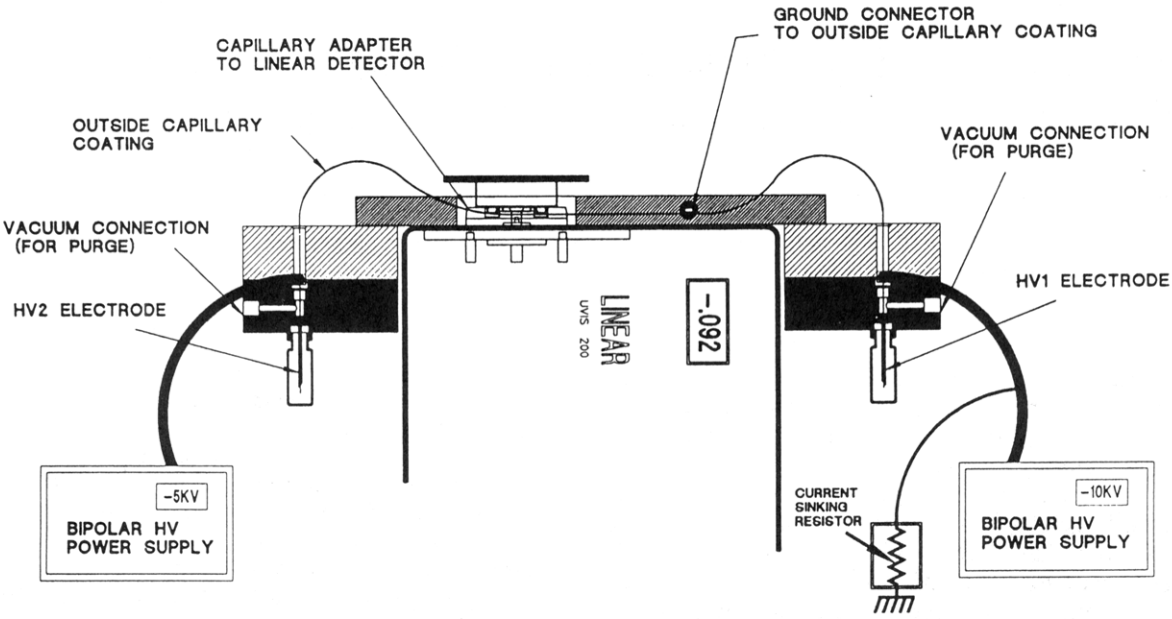

Figure 1. Experimental setup in an external grounding configuration for applying a radial electric potential gradient across the capillary wall (taken from ref 11).

the center of the capillary tubing was created by following the method of Lux et al.[12] Dual electric contacts at both sides of the UV window were made and then connected to ground in order to get a defined reference point. About 30% of the external surface of the capillary at the ends was left uncoated to avoid electric leakage between the grounded outside coating and the high-voltage (HV) electrodes.

For a full-range adjustment of electroosmosis, two HV power supplies were used. Because most HV power supplies were not designed to operate as current sinks, our experiments incorporated a resistor ladder in parallel to HV1 to facilitate the current sink function. On the basis of the external grounding, the electric field across the buffer solution in the capillary exerted a radial electric potential gradient upon the capillary wall. Since the applied radial electric potential gradient was varied along the capillary, an average radial electric potential gradient was calculated by averaging the radial electric potential gradients at each end of the capillary. The extent of electroosmotic control obtained by this external grounding configuration was comparable to those achieved by the coaxial configuration.[13]

Dimethyl sulfoxide and quinine hydrochloride in the solution mixture were used as the electroosmotic flow marker and the micellar marker, respectively.[14] The flow rate of electroosmosis was assigned as positive when the direction of flow was toward the cathodic end. The acetic acid buffer and dimethyl sulfoxide were purchased from Aldrich (Milwaukee, WI). Sodium dodecyl sulfate was obtained from Boehringer Mannheim GmbH (Germany). The PTH-amino acids, dodecyltrimethylammonium bromide (DTAB), and quinine hydrochloride were purchased from Sigma (St. Louis, MO).

The neutral PTH-amino acid mixture was prepared at a concentration of approximately 0.5 mg/mL for each amino acid. The separations of the PTH-amino acid mixture were conducted in a 40-cm-long capillary with a 50-μm i.d. and 150-μm o.d. (Polymicro Technologies Inc., Phoenix, AZ). The PTH-amino acid mixture was injected by using 2 kV for 10 s. A constant electric field equal to 200 V/cm was then applied for electrophoretic separation. For the separations of the PTH-amino acid mixture at various radial electric potential gradients, the anodic end of the electric field was always at the UV detector end. The separation distance between the injection point and the UV detector was 22.5 cm. The PTH-amino acids were monitored by a UV detector from Linear Instruments (Reno, NV) at 260 nm.

Table I. Effect of Radial Electric Potential Gradient on Electroosmotic Mobility in SDS Solution

potentials (kV)		av radial elec	μ_{eo}[a] (×10⁴	μ_{mc}[a] (×10⁴	
anode	cathode	potentl grad (kV)	cm²/V·s)	cm²/V·s)	$-t_{mc}/t_o$
−8	−16	+12	2.56	−2.44	1.05
−1	−9	+5	1.08	−3.94	0.27
+2	−6	+2	0.18	−4.84	0.04
+4	−4	0	0.38	−4.62	0.08
+8	0	−4	0.80	−4.20	0.19
+12	+4	−8	1.78	−3.22	0.55
+16	+8	−12	2.74	−2.28	1.20

[a] The experimental error in measuring the electroosmotic and micellar mobilities is about 3–5% for over five runs at various radial electric potential gradients.

RESULTS AND DISCUSSION

The electroosmotic mobility was about 0.96×10^{-4} cm²/V·s in a solution of 10 mM acetic acid at pH 3.6. With the addition of 50 mM SDS, the electroosmotic mobility was reduced to 0.38×10^{-4} cm²/V·s. When the solution pH was 3.6 in this study, the electrophoretic velocity of the SDS micelle in the direction toward the anodic end was greater than the electroosmotic velocity toward the cathodic end of the electric field. The SDS micelle monitored by quinine hydrochloride therefore migrated toward the anodic end, near the UV detector. As shown in Table I, the electroosmotic mobility, μ_{eo}, and the mobility of the SDS micelle, μ_{mc} (the summation of the micelle's electrophoretic velocity and the electroosmotic flow), were varied with the application of radial electric potential gradients. The mobilities summarized in Table I were assigned as positive when the direction of movement was toward the cathodic end of the electric field.

As summarized in Table I, the electroosmotic mobility was enhanced with the application of negative radial electric potential gradients. In the presence of positive radial electric potential gradients, the electroosmotic mobility was first reduced in the predicted manner, but then increased at higher positive radial electric potential gradients. Even when the solution buffer was changed from acetate to phosphate, the increase instead of decrease in the electroosmotic mobility

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Table II. Effect of Radial Electric Potential Gradient on Electroosmotic Mobility in DTAB Solution

av radial electric potential gradient (kV)	μ_{eo}[a] ($\times 10^4$ cm^2/V·s)
+6	−2.81
+4	−2.93
0	−3.00
−4	−3.08
−8	−3.13
−10	−3.22

[a] The experimental error in measuring the electroosmotic mobility is about 0–2% for over five runs at various radial electric potential gradients.

under the influence of positive radial electric potential gradients was observed again. The electrophoretic mobility of the SDS micelle, based on the difference between μ_{mc} and μ_{eo}, remained constant at various radial electric potential gradients.

There was no hysteresis in the direct control of electroosmosis by using a radial electric potential gradient across the capillary wall. The variation in surface coverage of adsorbed SDS onto the silica capillary was provided as a possible explanation for the increase in the electroosmotic mobility upon application of positive radial electric potential gradients. The surface charge at the capillary wall became less negative (or even positive) in the presence of positive radial electric potential gradients and then induced the stronger adsorption of negatively charged SDS at the capillary/solution interface. The apparent ζ potential and the resultant electroosmotic mobility were therefore increased with the application of positive radial electric potential gradients.

Due to the adsorption of DTAB at the capillary/solution interface, the electroosmotic mobility was reversed to −3.00 $\times$ 10^{-4} cm^2/V·s in a solution of 10 mM acetic acid/50 mM DTAB, pH 3.6. As summarized in Table II, the electroosmotic mobility in the absolute value was increased with the application of negative radial electric potential gradients. The surface charge at the capillary wall became more negative in the presence of negative radial electric potential gradients and then induced the stronger adsorption of positively charged DTAB at the capillary/solution interface. The apparent ζ potential therefore became more positive, and the resultant electroosmotic mobility toward the anodic end was increased with the application of negative radial electric potential gradients. In contrast, the surface charge at the capillary wall became less negative (or even positive) in the presence of positive radial electric potential gradients and then reduced the adsorption of positively charged DTAB on the capillary wall. The apparent ζ potential therefore became less positive and the anodic electroosmosis was decreased with the application of positive radial electric potential gradients. The range of electroosmotic control in DTAB solution with the application of various radial electric potential gradients was smaller than that observed in MEKC with the use of SDS. Again, there was no hysteresis in the direct control of electroosmosis by using a radial electric potential gradient across the capillary wall.

As demonstrated by Terabe et al.,[15] neither organic additives nor pH of the solution was effective in altering electroosmosis and retention window in MEKC with the use of SDS. In the presence of SDS, the polymer coating on the inner wall of the capillary tubing dramatically changed electroosmosis: the polyethylene glycol coating made electroosmosis weaker and the methyl silicone stronger than the untreated surface.[15] By simply changing the radial electric potential gradient from 0 to −12 kV, the ratio of $-t_{mc}$ to t_o as the retention window summarized in Table I was increased from 0.08 to 1.20 in a solution of 10 mM acetic acid/50 mM SDS, pH 3.6. There was no additional solute dispersion and band broadening induced by the direct control of electroosmosis and retention window. The increase in the retention window with the application of negative radial electric potential gradients would thus enhance the separation resolution in MEKC.

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Table III. Elution Order and Capacity Factor for PTH-Amino Acids Examined in This Study

PTH-amino acid	elution order	capacity factor[a]
PTH-phenylalanine	1	8.23
PTH-tryptophan	2	7.17
PTH-isoleucine	3	6.55
PTH-methionine	4	3.59
PTH-valine	5	3.44
PTH-tyrosine	6	2.41
PTH-alanine	7	1.28
PTH-glycine	8	0.81
PTH-serine	9	0.55
PTH-threonine	10	0.48

[a] The experimental error in measuring the capacity factor is about 3% for over five runs.

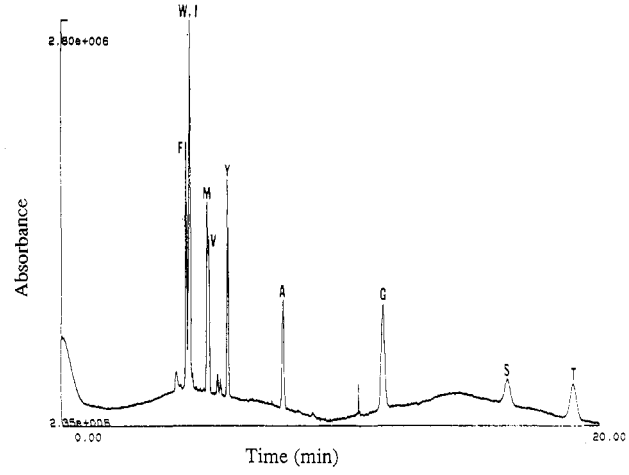

Figure 2. Electrophoretic separation of PTH-amino acids in the presence of a 0-kV radial electric potential gradient: F, phenylalanine; W, tryptophan; I, isoleucine; M, methionine; V, valine; Y, tyrosine; A, alanine; G, glycine; S, serine; T, threonine. Buffer, 10 mM acetic acid/50 mM SDS, pH 3.6; capillary, 50-μm i.d. and 150-μm o.d., length to detector 22.5 cm; voltage, 2 kV and 10 s for injection, 8 kV for electrophoresis.

To investigate the effect of electroosmotic control on the separation resolution in MEKC, the separations of neutral PTH-amino acids as the model system were examined in a 50-μm-i.d. and 150-μm-o.d. capillary with a solution of 50 mM SDS/10 mM acetic acid, pH 3.6. The PTH-amino acid mixture was injected at the cathodic end and detected near the anodic end of capillary. The elution order and the capacity factor measured from single component analysis were summarized in Table III for more hydrophobic amino acids eluting earlier with the micelle phase. The capacity factor of PTH-amino acids all remained constant at various radial electric potential gradients.

As shown in Figure 2, phenylalanine as peak 1 was almost baseline resolved and separated from tryptophan and isoleucine as peaks 2 and 3 in the presence of a 0-kV radial electric potential gradient. However, the tryptophan/isoleucine and methionine/valine pairs of peaks were poorly resolved due to the small difference in their capacity factors. All 10 neutral PTH-amino acids were eluted within 20 min

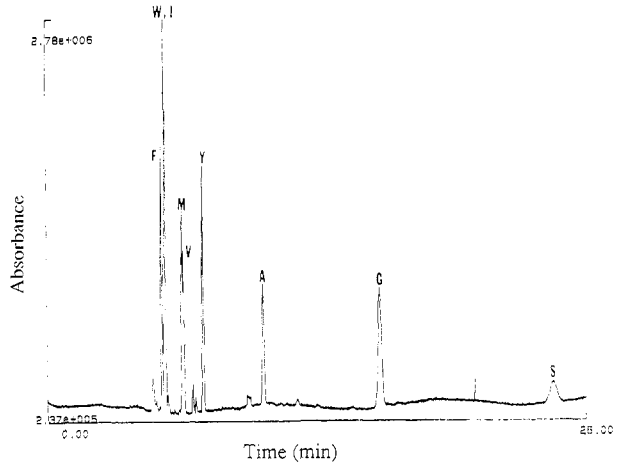

Figure 3. Electrophoretic separation of PTH-amino acids in the presence of a −4-kV radial electric potential gradient. Other conditions are the same as in Figure 2.

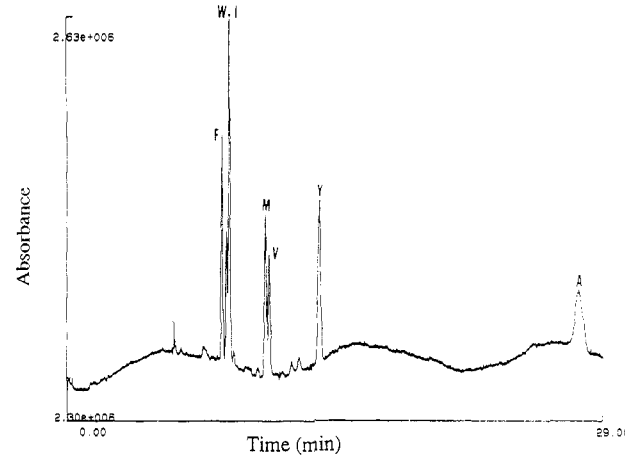

Figure 4. Electrophoretic separation of PTH-amino acids in the presence of a −8-kV radial electric potential gradient. Other conditions are the same as in Figure 2.

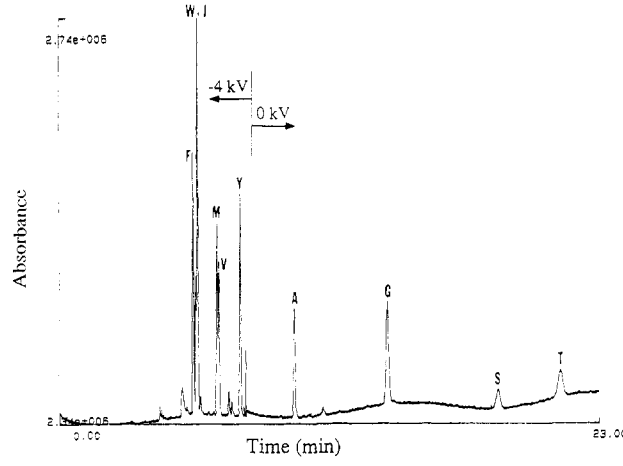

Figure 5. Electrophoretic separation of PTH-amino acids in the presence of a dynamic radial electric potential gradient between −4 and 0 kV. Other conditions are the same as in Figure 2.

of the separation time. In addition, there were several impurity peaks associated with the PTH-amino acids of methionine, valine, and tyrosine.

With the application of a −4-kV radial electric potential gradient as shown in Figure 3, the enhanced electroosmotic flow in the direction against the electrophoretic migration of the SDS micelle resulted in an increase of the migration time of amino acids in the capillary. The increase in the separation resolution of amino acids was observed especially between phenylalanine as peak 1 and tryptophan/isoleucine as peaks 2 and 3. However, threonine as the last amino acid in the mixture was not eluted and observed within 30 min of the separation time.

As shown in Figure 4, significant improvement of the separation resolution for PTH-amino acids was clearly observed upon the application of a −8-kV radial electric potential gradient. Tryptophan and isoleucine as peak 2 and peak 3 and methionine and valine as peak 4 and peak 5 were almost baseline separated and resolved. The difference in the capacity factor between methionine and valine was only 0.15. This separation resolution was obtained, however, at a large expense in the analysis time. For example, it took nearly 30 min of the separation time just for the elution of alanine as peak 7.

In order to optimize both the separation efficiency and resolution in MEKC, the dynamic control of electroosmosis and retention window during the separation is currently under investigation. As shown in Figure 5, the applied radial electric potential gradient was changed from −4 to 0 kV right after the elution of tyrosine as peak 6. All 10 amino acids were eluted and observed within 25 min of the separation time in a dynamic control of electroosmosis. By dynamically varying the range of retention window during the separation, the separation resolution for the first five amino acids was enhanced without a dramatic increase in the elution times for the later eluting peaks. The preliminary result shown in Figure 5 clearly demonstrates a great potential in the application of an electroosmotic gradient elution for the MEKC separations.

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