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Infinite Elution Range in Micellar Electrokinetic Capillary Chromatography Using a Nonionic/Anionic Mixed Micellar System

Eric S. Ahuja, Edward L. Little,[†,‡] Kurt R. Nielsen,[§] and Joe P. Foley*

Department of Chemistry, Villanova University, Villanova, Pennsylvania 19085

The feasibility of increasing the elution range in micellar electrokinetic capillary chromatography (MEKC) was investigated using a nonionic/anionic mixed micellar system. The micellar "pseudostationary" phase was comprised of a mixture of nonionic (poly(oxyethylene)(23) dodecanol) and anionic (sodium dodecyl sulfate) surfactants. Variation in poly(oxyethylene)(23) dodecanol (Brij 35, Aldrich) concentration affected the electroosmotic and micellar electrophoretic mobilities differently. Equal but opposite electroosmotic and micellar electrophoretic velocities were obtained at a specific Brij 35 concentration, thereby producing a "stationary" micellar phase. Retention characteristics of a series of *n*-alkylphenone homologues suggested a nearly infinite elution range. Infinite elution ranges are possible at neutral pH (7.0) using deactivated fused-silica capillaries and at a pH of 6.2 using untreated fused-silica capillaries.

Micellar electrokinetic capillary chromatography (MEKC)[1,2] has grown significantly since its inception due to its ability to provide high-efficiency separations for a wide variety of neutral solute systems. The mechanism behind separations in MEKC is the differential partitioning of solutes between an aqueous phase and a micellar "pseudostationary" phase. Anionic surfactant systems are typically used in MEKC since the resultant micelles electrophoretically migrate opposing electroosmotic flow and do not interact appreciably with the negatively charged walls of the fused-silica capillary columns. Sodium dodecyl sulfate (SDS) is by far the most popular micellar system in MEKC due to its low cost, availability in high purity, UV absorption characteristics, and intrinsic micellar properties.

Although anionic surfactants have received the bulk of attention to date, their usefulness is limited by the concentrations which may be utilized in MEKC. Due to its charge, high concentrations of SDS induce significant Joule heating, which consequently degrades separation efficiency. The use of nonionic surfactants or other net zero charge (NZC) surfactants in MEKC may be advantageous since micellar concentration can be increased dramatically with no change in operating currents at constant voltage. However, anionic surfactant must be added to the nonionic micellar system to form mixed micelles since nonionic micelles lack sufficient surface charge to electrophoretically migrate. This presence of SDS in the mixed micelles results in their migration against the bulk electroosmotic flow. The general advantages of NZC surfactants in combination with anionic surfactants to form mixed micelles for use as pseudostationary phases in MEKC have recently been reported.[3]

Poly(oxyethylene)(23) dodecanol (Brij 35, Aldrich) has been demonstrated in previous studies to be useful as a pseudostationary phase in MEKC. Initial studies of this micellar system showed particular promise in alteration of selectivity; benzene and benzaldehyde, inseparable in SDS or STS (sodium decyl sulfate), were easily separated using a Brij 35/SDS micellar phase.[4] Our group has further demonstrated the usefulness of this micellar system in the optimization of solute resolution via changes in surfactant concentration.[5]

One of the primary drawbacks of MEKC is the limited time span where solute elution may occur, and the extension of the elution range has been the topic of several studies. Otsuka and Terabe[6] illustrated the effects of pH on electrokinetic velocities. Electroosmosis decreased regularly with decreasing pH, while the electrophoretic velocity of the micelles remained virtually constant. Consequently, the electroosmotic and SDS micellar electrophoretic velocities were nearly equivalent at pH 5.0. Adjustment of the micellar solution pH to 5.0 would therefore provide a substantial increase in elution range. Balchunas and Sepaniak[7] investigated the use of surface-silanated fused-silica capillaries to reduce electroosmotic flow. This technique was successful in extending the elution range and provided t_{mc}/t_o values of approximately 10. Unfortunately, the increase in elution range reported in this study also came at a substantial increase in analysis time, with some separations taking almost 100 min. In addition, the running buffer pH was not reported, thus making it difficult to reproduce this study. Finally, a loss in column efficiency due to solute–wall interactions with the silanated capillaries required the addition of 2-propanol to the mobile phase to limit these types of interactions and improve the mass-transfer kinetics resulting in improved column efficiencies. The use of 2-propanol increases the already long analysis times and from this viewpoint is not advantageous. Increased t_{mc}/t_o values have been observed with other organic solvent modification such acetonitrile and methanol, but the magnitude of such increases is rather small.

[†] Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803.

[‡] Present address: Columbian Chemicals Co., P.O. Box 96, Swartz, LA 71281.

[§] Present address: Bachem Bioscience Inc., 3700 Horizon Dr., King of Prussia, PA 19406.

(1) Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Anal. Chem.* **1984**, *56*, 111–113.

(2) Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1985**, *57*, 834–841.

(3) Ahuja, E. S.; Preston, B. P.; Foley, J. P. *J. Chromatogr.* **1994**, 657, 271–284.

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(7) Balchunas, A. T.; Sepaniak, M. J. *Anal. Chem.* **1987**, *59*, 1466–1470.

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At this time, only limited elution range extension is possible using organic cosolvents.

Each of the previous studies focused on increasing the elution range by adjustment of electroosmotic flow via modification of the fused-silica surface. Mixed NZC/anionic micellar systems may facilitate the control (increase) of the elution range by adjustment of the micellar electrophoretic mobility rather than the electroosmotic velocity. In this investigation, we present our findings on the usefulness of a Brij 35/SDS micellar phase in achieving an infinite elution range in MEKC.

EXPERIMENTAL SECTION

Apparatus. A Waters Quanta 4000 capillary electrophoresis system (Waters Inc., Milford, MA) equipped with fixed-wavelength UV detection at 254 nm was employed for all the separations performed in this study. All MEKC separations were performed in either a 30-cm (injection to detection) × 50-μm-i.d. (370-μm-o.d.) untreated fused-silica capillary (Polymicro Technologies, Tucson, AZ) or a deactivated (trimethylchlorosilane (TMCS) treated) fused silica capillary (Alltech Associates, Inc., Deerfield, IL) unless otherwise noted in the text. A window was burned through the polyimide coating at a distance of 7.5 cm from the outlet end of each capillary, yielding a capillary with injector-to-detector length of 30.0 cm. The total capillary length was 37.5 cm. Injections were made hydrostatically for 1 s. The applied voltage was 25 kV, and operating currents were less than 35 μA unless otherwise noted in the text. The data were collected at a rate of 20 points/s and analyzed on a Macintosh IIci computer (Apple, Cupertino, CA) using a MacLab 4 channel ADC with the appropriate vendor software (ADInstruments, Milford, MA). All experiments were done at ambient temperature (~25 °C).

Materials. The *n*-alkylphenone homologous series was purchased as a kit from Aldrich (Milwaukee, WI) and consisted of C_8 (acetophenone), C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{16}, and C_{18} (dodecanophenone) homologues. Sodium dodecyl sulfate (SDS) was purchased from Sigma (St. Louis, MO), while Brij 35 (poly(oxyethylene)(23) dodecanol) was purchased from Aldrich. All surfactants were used as received. The concentration of SDS was either 20 or 40 mM for all the MEKC separations. The concentration of Brij 35 was varied from 2–60 mM. Stock buffer solutions were prepared with $NaH_2PO_4{\cdot}H_2O$ and sodium hydroxide (NaOH) to give a 100 mM phosphate buffer at pH 7.0 for runs performed with the silanated capillary, while runs done with the untreated fused-silica capillary were done at pH values of 7.0 and 6.2. A phosphate buffer concentration of 10 mM was used in all the experiments. The micellar solutions were made by weighing appropriate amounts of SDS or Brij 35 and diluting with the stock buffer solution and distilled water in a 100-mL volumetric flask to obtain the desired concentrations. All the micellar buffer solutions were filtered through 0.20-μm membrane filters obtained from Alltech. HPLC grade distilled water used in the makeup of the micellar buffer solutions was obtained from J. T. Baker (Phillipsburg, NJ). Sample solutions were made up of 50% acetonitrile (MeCN) and 50% running buffer, with solute concentrations at or below 0.75 mg mL^{-1}.

Procedures. Activation of the capillaries was performed using a modification of a procedure described previously.[8] The capillary was initially rinsed with 1 M KOH for 20 min, followed by subsequent rinses of 0.1 M KOH and distilled water for 20 min each. The capillary was then rinsed for 20 min with the operating buffer. Purges with the operating buffer were performed after each run for 5–10 min using a vacuum of ~16.5 in. of Hg at the detector reservoir.

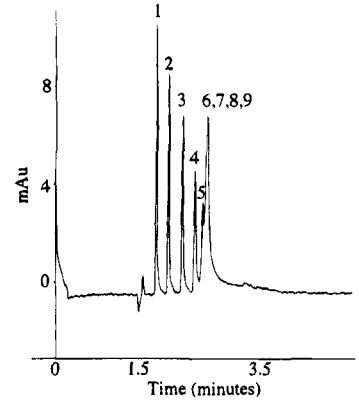

Figure 1. Electrokinetic chromatogram of *n*-alkylphenone homologues obtained with an untreated fused-silica capillary. Peak identity: (1) acetophenone (C_8); (2) propiophenone (C_9); (3) butyrophenone (C_{10}); (4) valerophenone (C_{11}); (5) hexanophenone (C_{12}); (6) heptanophenone (C_{13}); (7) octanophenone (C_{14}); (8) decanophenone (C_{16}); and (9) dodecanophenone (C_{18}). Capillary: 50 μm i.d., 370 μm o.d., and 37.5 cm length (30.0 cm to detector). Detection wavelength was 254 nm. Micelle medium was 0.010 M Brij 35/0.020 M SDS in 10 mM phosphate buffer (pH 7.0); applied voltage was 25 kV.

The electroosmotic and net micellar velocities, ν_{eo} and ν_{mc}, were determined from the elution times of acetonitrile, t_o,[9] and the longest-chain *n*-alkylphenone homologue (C_{18}), t_{mc}, respectively, by the relationship $\nu = L/t$, where L is the injector-to-detector column length. Micellar electrophoretic velocities, ν_{ep}, were calculated by the following relationship:

$$\nu_{ep} = \nu_{eo} - \nu_{mc} \quad (1)$$

RESULTS AND DISCUSSION

In this investigation, two different types of fused-silica capillaries were used, a deactivated fused-silica capillary which was end-capped with trimethylchlorosilane and an untreated fused-silica capillary. The latter are by far the most common type of capillaries used in MEKC. The use of surface-silanated capillaries is not as common. Even though the use of silanated capillaries has been shown to induce greater solute–wall interactions and lower separation efficiencies, the reduction in electroosmotic flow with the use of silanated capillaries can be advantageous.[7] For these reasons, both types of fused-silica capillaries were employed in this study.

Figure 1 shows a typical MEKC chromatogram of *n*-alkylphenone homologues. As was stated in the introduction, MEKC is limited by the time in which a separation must occur which is specifically caused by the lack of a true stationary phase. The micellar phase in MEKC serves as a pseudostationary phase, permitting the separation of solutes within a limited time frame. Resolution, R_s, in MEKC is described by

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(9) Ahuja, E. S.; Little, E. L.; Foley, J. P. *J. Liq. Chromatogr.* **1992**, *14*, 145–155.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{1+k}\right)\left(\frac{1 - t_o/t_{mc}}{1 + (t_o/t_{mc})k}\right) \quad (2)$$

where N is the column efficiency, α is the selectivity, k is the retention factor, and t_o and t_{mc} are the elution times of an analyte unretained and infinitely retained by the micelle, respectively. The factor t_{mc}/t_o describes the elution range of the separation.

In MEKC, it is common practice to work under conditions where the electroosmotic velocity is greater than the electrophoretic velocity of the micelles in the opposite direction. Therefore, the micelles are not infinitely retained within the capillary but elute in a finite period of time. It is obvious that the possibility of two solutes being resolved from one another is controlled not only by the column efficiency, separation selectivity, and degree of retention by the micellar phase but also by the relative amount of time these solutes experience the separation mechanism. It is therefore desirable to work under conditions where the elution range is as long as possible.

Note that for an infinite value of t_{mc}/t_o ($t_o/t_{mc} = 0$), the last term of eq 2 equals unity and solute resolution is consequentially maximized. This condition is possible only if the net micellar velocity, v_{mc}, is 0, that is, $v_{eo} = v_{ep}$ (see eq 1). In other words, the micellar phase would be truly stationary within the column. Previous studies have concentrated solely on decreasing the electroosmotic velocity to produce larger elution ranges. This approach is not particularly advantageous, however, since a gross reduction in electroosmotic flow lengthens analysis times and degrades column efficiency.[10] It would therefore be more desirable to increase the electrophoretic velocity of the micelles by modifying the micellar phase while keeping the electroosmotic flow as high as possible. However, the electroosmotic velocity (in one direction) *must* be less than the electrophoretic velocity of the micellar phase (in the opposite direction) in order to equate the two velocities by micellar phase modification.

Table 1 gives a comparison of electroosmotic and micellar electrophoretic velocities for two surfactant systems, pure SDS and mixed Brij 35/SDS, obtained with the surface-silanated capillary. The electroosmotic velocity, v_{eo}, is defined as

$$v_{eo} = \frac{\epsilon \zeta_{cap}}{\eta} E \quad (3)$$

where ϵ and η are the dielectric constant and viscosity of the solution, respectively, ζ_{cap} is the ζ-potential at the solid–liquid interface of the capillary wall and solution, and E is the electrical field strength, equivalent to applied voltage per unit length. A similar expression can be given for the micellar electrophoretic velocity, v_{ep},

$$v_{ep} = \frac{2\epsilon \zeta_{mc}}{3\eta} f(\kappa a) E \quad (4)$$

where ζ_{mc} is the ζ-potential at the *micellar* surface and $f(\kappa a)$ is a function dependent on the micellar size and shape. As previously discussed, operating conditions were such that the electroosmotic velocity was greater than the electrophoretic velocity for each micellar system, producing chromatographic separations similar to those in Figure 1. Greater velocities were observed in the SDS systems than in Brij 35/SDS, even though the applied voltage and, hence, electrical field strength were less. This was probably due to the lower inherent viscosity of pure SDS solutions over their chromatographically equivalent Brij 35/SDS counterparts, reduced even further by the greater Joule heating in the SDS systems, which results in higher temperatures and still lower viscosities. We observed that the Brij 35/SDS solutions were more viscous than pure SDS solutions. However, these observations were purely qualitative, and we did not attempt to measure viscosities or to find support for our observations in the literature.

Table 1. Comparison of Electroosmotic and Micellar Electrophoretic Velocities in Anionic and Nonionic/Anionic Micellar Systems[a]

[SDS], M	v_{eo}	μ_{eo}	$\lvert v_{ep}\rvert$	μ_{ep}
0.040	1.805	0.086	0.917	0.044
0.060	1.799	0.085	0.920	0.044
0.080	1.806	0.086	0.938	0.045
0.100	1.736	0.082	0.941	0.045
[Brij 35], M[b]	v_{eo}	μ_{eo}	$\lvert v_{ep}\rvert$	μ_{ep}
0.030	0.858	0.016	0.490	0.009
0.040	0.758	0.014	0.381	0.007
0.050	0.618	0.012	0.297	0.006
0.060	0.531	0.010	0.240	0.005

[a] Velocities are in units of mm/s. Mobilities are in units of $mm^2/V\cdot s$. Applied voltages: SDS, 10 kV; Brij 35/SDS, 25 kV. [b] Brij 35/SDS mixed surfactant system in which [Brij 35] is varied while [SDS] is maintained at 0.020 M.

In the SDS surfactant system, the electroosmotic and micellar electrophoretic velocities remained fairly constant over the [SDS] range which was studied. For the Brij 35/SDS systems, both velocities decreased significantly with increasing Brij 35 concentration. Since 0.020 M SDS was present in each Brij 35/SDS micellar system, Joule heating should have been nearly equivalent for each [Brij 35] variation.

It was expected that the micellar electrophoretic velocity would change with [Brij 35]. At constant [SDS], Brij 35/SDS micelles should become increasingly less ionic as [Brij 35] increases. This reduction of SDS within individual micelles would decrease their surface charge density, producing lower electrophoretic mobility. Serendipitously, the electroosmotic velocity also decreased with increasing [Brij 35]. Three factors may have caused this decrease in v_{eo}: (i) an increase in solution viscosity, (ii) adsorption of Brij 35 to the capillary surface, or (iii) a combination of the two. Brij 35/silica surface adsorption has been discussed from a chromatographic standpoint by Towns and Regnier,[11] where they witnessed a decrease in v_{eo} with increasing [Brij 35] at concentrations above and below the CMC. However, their work employed surface-silanated capillaries which had not been base-activated after silanation with octadecyltrichlorosilane and is therefore not directly applicable to our study. It is important to note that we used surface-silanated capillaries that were base-activated. Even though we base-activated the capillaries used in this study, it is likely that some TMCS silanation was still present on the capillary surface. The results reported in Table 1 are most probably a combination of the adsorption of Brij 35 to the capillary wall and

(10) Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1989**, *61*, 251–260.

(11) Towns, J. K.; Regnier, F. E. *Anal. Chem.* **1991**, *63*, 1126–1132.

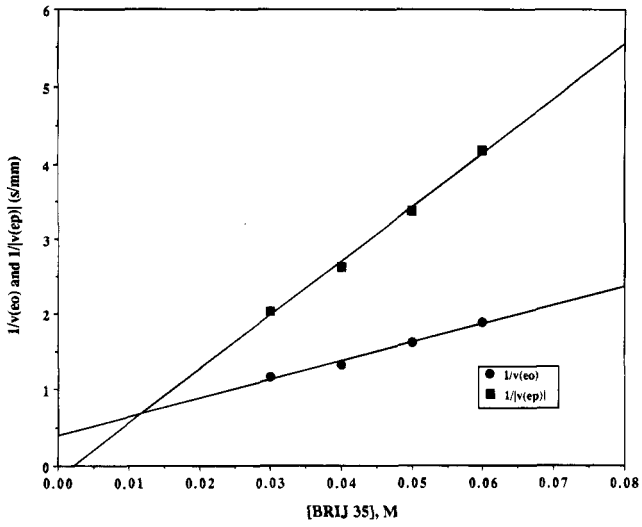

Figure 2. Dependence of electroosmotic velocity and the electrophoretic velocity of the Brij 35/SDS mixed micelle on [Brij 35] for the surface-silanated fused-silica capillary. Operating conditions are given in the Experimental Section.

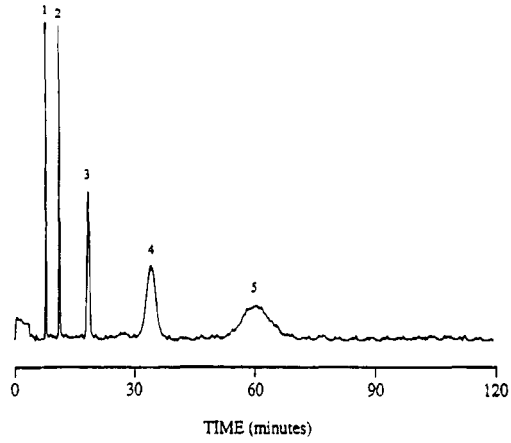

Figure 3. Electrokinetic chromatogram of *n*-alkylphenone homologues with surface-silanated fused-silica capillary under infinite elution range conditions. See Figure 1 for peak identities. Capillary: 50 μm i.d., 370 μm o.d., and 47.5 cm length (40.0 cm to detector). Detection wavelength was 254 nm. Micelle medium was 0.0118 M Brij 35/0.020 M SDS in 10 mM phosphate buffer (pH 7.0); applied voltage was 25 kV.

an increase in solution viscosity. Rinsing the capillary with phosphate buffer for 5–10 min between each run was effective in reducing the buildup of Brij 35 on the capillary wall. In our case, we believe that adsorption processes are not significant and/or reach equilibrium conditions rather rapidly as evidenced by good run-to-run repeatability at individual Brij 35 concentrations. Therefore, we maintain that viscosity variation with Brij 35 concentration is the primary contributor to the observed decrease in ν_{eo}. It is also likely that the variation in viscosity affects ν_{ep} since it, too, is viscosity dependent. However, this effect should be additive to the velocity change resulting from modification of micellar electrophoretic mobility.

Figure 2 illustrates the change in reciprocal velocities with Brij 35 concentration for the Brij 35/SDS systems in Table 1. The most interesting aspect of Figure 2 is the intersection of the lines at ~0.012 M Brij 35. Clearly, the electroosmotic and micellar electrophoretic velocities should be equal at the point of intersection, assuming that no deviations from linearity occur at Brij 35 concentrations lower than 0.030 M. Thus, the elution range (designated by t_{mc}/t_o) should be infinite at these conditions.

Figure 3 shows a chromatogram of *n*-alkylphenones using a micellar phase consisting of 0.020 M SDS and 0.0118 M Brij 35, the concentration at the intersection of the curves in Figure 2. Several sample components including the t_{mc} marker (C_{18}) did not elute in the length of time allotted for this analysis (120 min). In fact, the last peak to elute from the column, C_{12}, required more than 60 min. Note also that the elution time for acetonitrile, the t_o marker, was ~4.7 min, as denoted by the baseline depression toward the beginning of the chromatogram. Using the analysis time as a lower limit for t_{mc}, this particular chromatogram represents an elution range (t_{mc}/t_o) in excess of 25, significantly higher than any reported t_{mc}/t_o ratio to date, especially those obtained at a relatively high pH (pH ≥ 7.0).

With regard to the apparently rapid loss in column efficiency with increasing solute retention shown in Figure 3, we have two comments.

First, much of the apparent loss in efficiency is a result of the use of on-column detection, the preferred detection format for most spectroscopic detectors in CZE and MEKC. As noted by Hjertén et al.[12] and Zare et al.,[13] on-column detection causes solutes that migrate more slowly through the column to appear artificially broad relative to analytes that migrate faster. Although not widely appreciated, peak widths should be corrected for zone velocity via eq 5 before column efficiencies or comparing zone widths of early and late-eluting peaks are calculated:

$$W_{corr} = \frac{L_d}{t_R} W_{obs} \qquad (5)$$

Here, L_d, t_R, W_{obs}, and W_{corr} are the length of capillary from injection to detector, the migration time of the analyte, and the observed and corrected widths of the analyte peak, respectively. When this correction is made to the data of Figure 3, only the expected gradual decrease in N with increasing k[10,14] is observed.

Second, although the apparent gross variation in column efficiency over time could limit the applicability of the infinite elution range format in MEKC, this problem could be minimized to a satisfactory level by optimizing the experimental conditions (e.g., by using a shorter column and/or a higher field strength). Moreover, the capability to observe changes in column efficiency over such a wide time frame could be used advantageously in investigations of band-broadening mechanisms. Clearly, the use of nonionic/anionic micellar phases in MEKC to control the elution range should be as useful in the precise evaluation of solute band-broadening as a previously reported method by Terabe et al. using back-and-forth electrophoresis,[15] especially since electrical field perturbations caused by reversing voltage polarity are avoided.

Initially, evaluation of the untreated fused-silica capillaries was done at pH 7.0. Table 2 lists the electroosmotic and micellar

(12) Hjertén, S.; Elenbring, K.; Kilár, F.; Liao, J.-L.; Chress, A. J. C.; Sierbert, C. J.; Zhu, M.-D. *J. Chromatogr.* **1987**, *403*, 47.
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(14) Davis, J. M. *Anal. Chem.* **1989**, *61*, 2455–2461.
(15) Terabe, S.; Shibata, O.; Isemura, T. *J. High Resolut. Chromatogr.* **1991**, *14*, 52–55.
(16) Lukacs, K. D.; Jorgenson, J. W. *J. High Resolut. Chromatogr., Chromatogr. Commun.* **1985**, *8*, 407.

Table 2. Comparison of Electroosmotic and Micellar Electrophoretic Velocities and Mobilities with the [Brij 35]/0.020 M SDS Micellar Systems[a]

capillary	pH	[Brij 35] (M)	v_{eo} (mm/s)	μ_{eo} (mm^2/V·s)	v_{ep} (mm/s)	μ_{ep} (mm^2/V·s)
untreated fused-silica	7.0	0.002	4.082	0.061	3.120	0.047
		0.010	3.891	0.058	1.750	0.026
		0.020	3.257	0.049	1.030	0.015
		0.030	2.841	0.043	0.720	0.011
		0.040	1.852	0.028	0.560	0.008
		0.050	0.982	0.015	0.380	0.006
		0.060	0.743	0.011	0.310	0.005
untreated fused-silica	6.2	0.020	1.331	0.020	1.143	0.017
		0.030	0.951	0.014	0.799	0.012
		0.050	0.588	0.009	0.465	0.007
		0.060	0.496	0.007	0.384	0.006
silanated fused-silica	7.0	0.030	1.193	0.018	0.821	0.012
		0.040	0.929	0.014	0.615	0.009
		0.050	0.772	0.012	0.549	0.008
		0.060	0.639	0.010	0.383	0.006

[a] The applied voltage was 25 kV.

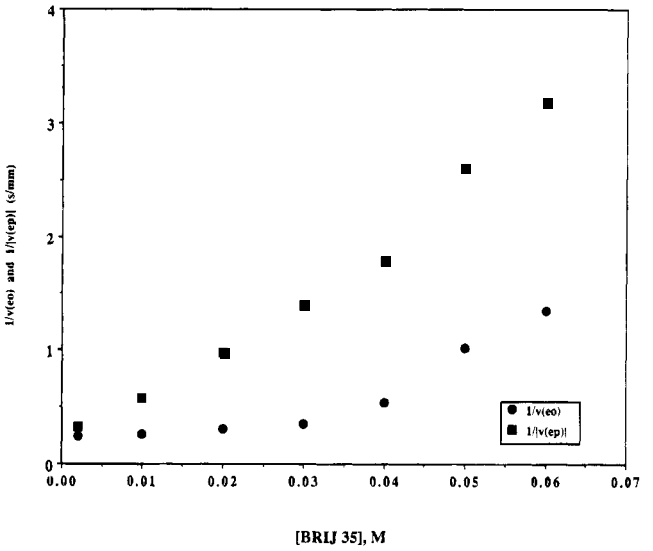

Figure 4. Dependence of electroosmotic velocity and the electrophoretic velocity of the Brij 35/SDS mixed micelle on [Brij 35] for the untreated fused-silica capillary at pH 7.0. Operating conditions are given in the Experimental Section.

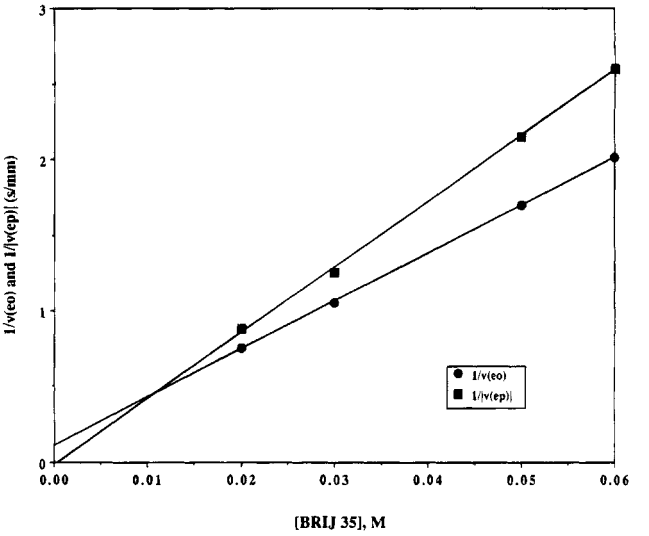

Figure 5. Dependence of electroosmotic velocity and the electrophoretic velocity of the Brij 35/SDS mixed micelle on [Brij 35] for the untreated fused-silica capillary at pH 6.2. Operating conditions are given in the Experimental Section.

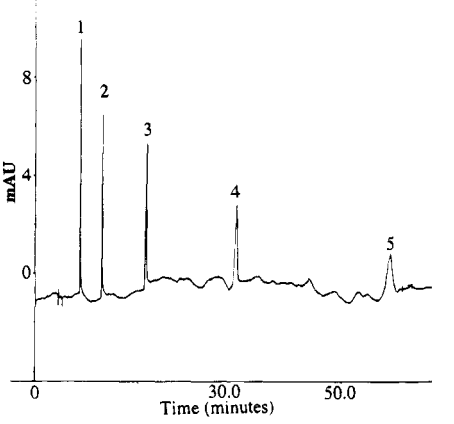

Figure 6. Electrokinetic chromatogram of *n*-alkylphenone homologues with the untreated fused-silica capillary at infinute elution range conditions. See Figure 1 for peak identities. Capillary: 50 μm i.d., 370 μm o.d., and 37.5 cm length (30.0 cm to detector). Detection wavelength was 254 nm. Micelle medium was 0.012 M Brij 35/0.020 M SDS in 10 mM phosphate buffer (pH 6.2); applied voltage was 25 kV.

electrophoretic velocities in the [Brij 35]/0.020 M SDS micellar systems for both types of fused-silica capillaries. The column length was shortened to 30 cm (injection to detection) in an attempt to generate higher column efficiencies and shorter analysis times. Figure 4 displays the plot of reciprocal electroosmotic and micellar electrophoretic velocities for the untreated fused-silica capillary at pH 7.0. It appears from Figure 4 that an infinite elution range may be possible at a Brij 35 concentration of ~ 0.001–0.002 M. However, separations performed with 0.002 M Brij 35 resulted in a t_{mc}/t_o value of 4.32. At this pH, the electroosmotic velocity was too high to obtain an infinite elution range. An effective way to reduce the electroosmotic velocity is to lower the pH.[16] Table 2 also lists the electroosmotic and micellar electrophoretic velocities for the untreated fused-silica capillary at pH 6.2. Figure 5 displays the reciprocal electroosmotic and micellar electrophoretic velocities as function of Brij 35 concentration at this pH. The intersection of the lines at a Brij 35 concentration of approximately 0.012 M indicates that a mixed micellar solution consisting of 0.012 M Brij 35/0.020 M SDS, under our experimental conditions, should yield an infinite elution range. Figure 6 shows the separation of alkylphenone homologues at this concentration. As was the case with the surface-silanated capillary, only the first five homologues eluted from the capillary in the allotted analysis time. One advantage in using the untreated fused-silica capillary for obtaining infinite elution ranges with the Brij 35/SDS micellar system over the silanated capillary is the sharper peaks that are generated, especially for the more hydrophobic solutes. This is easily seen if one compares peak 5 in Figure 3 with that obtained in Figure 5. We observed the runs to be more repeatable with the untreated fused-silica capillary than with the surface-silanated capillary. This is most probably due to decreased adsorption of the Brij 35 surfactant on the capillary surface since there are no alkyl groups directly attached to the wall of the capillary as found with the surface-silanated capillary. In addition, rinsing the untreated fused-silica capillary with phosphate buffer between analyses further diminished the buildup of surfactant at the capillary wall. A decrease in wall adsorption

of the surfactant would mean less on-column band-broadening and the generation of sharper peaks which was observed with the untreated fused-silica capillary.

It is very important to rinse either type of capillary between each analysis with the buffer solution (without surfactant) employed. The capillary rinse (purge) time for our study was 5 min, but this will vary depending on the length of capillary used. In general, we found that the silanated capillaries required longer purge times between analyses than did the untreated fused-silica capillaries. For high concentrations of Brij 35 (>0.030 M) used with the silanated capillaries, a minimum purge time of 10 min was needed to obtain repeatable runs. A 5-min purge of the untreated fused-silica capillary was sufficient in our case to provide repeatable analyses.

Although the time required to perform a separation increased dramatically using this mixed micellar system, this did not guarantee an infinite elution range with either type of capillary. To gain further insight into the magnitude of the elution range, we calculated retention factors for the *n*-alkylphenone homologues using the relationship between k and retention time in conventional LC

$$k = \frac{t_R - t_o}{t_o} \tag{6}$$

where t_R is the retention time of a solute of interest. This equation contrasts with the usual expression for k in MEKC:

$$k = \frac{t_R - t_o}{t_o(1 - t_R/t_{mc})} \tag{7}$$

It is well-known that a plot of log k versus carbon number should be linear for a homologous series.[17,18] We have observed such linearity for *n*-alkylphenones in MEKC under conditions where eq 7 was used to determine k.[19] If an infinite elution range was achieved in MEKC, then a linear relationship should be observed if capacity factors are calculated from the k expression in conventional LC (eq 6). Any deviation from linearity would be obvious for later eluting homologues since the additional term in the denominator of eq 6 becomes more significant as solute retention increases.

Figure 7 shows log k versus carbon number plots for *n*-alkylphenones using the data of Figure 6 (near-infinite elution range, t_{mc}/t_o > 17) and Figure 1 (finite elution range, t_{mc}/t_o = 1.91). Importantly, capacity factors for both curves in Figure 7 were calculated according to eq 6 (conventional LC) rather than eq 7 (MEKC). The plot in Figure 7 (top) of the data from Figure 1 (t_{mc}/t_o = 1.91) shows the curvature that is expected when an equation that assumes an infinite elution range, eq 6, is used to calculate retention factors obtained under finite elution range conditions. As shown here, deviations in linearity resulting from a finite elution range are readily discernible. In contrast, the plot in Figure 7 (bottom) of the data from Figure 6 (t_{mc}/t_o > 17) is perfectly linear (r^2 = 1.000), implying a near infinite elution range for the 0.012 M Brij 35/0.020 M SDS surfactant system under

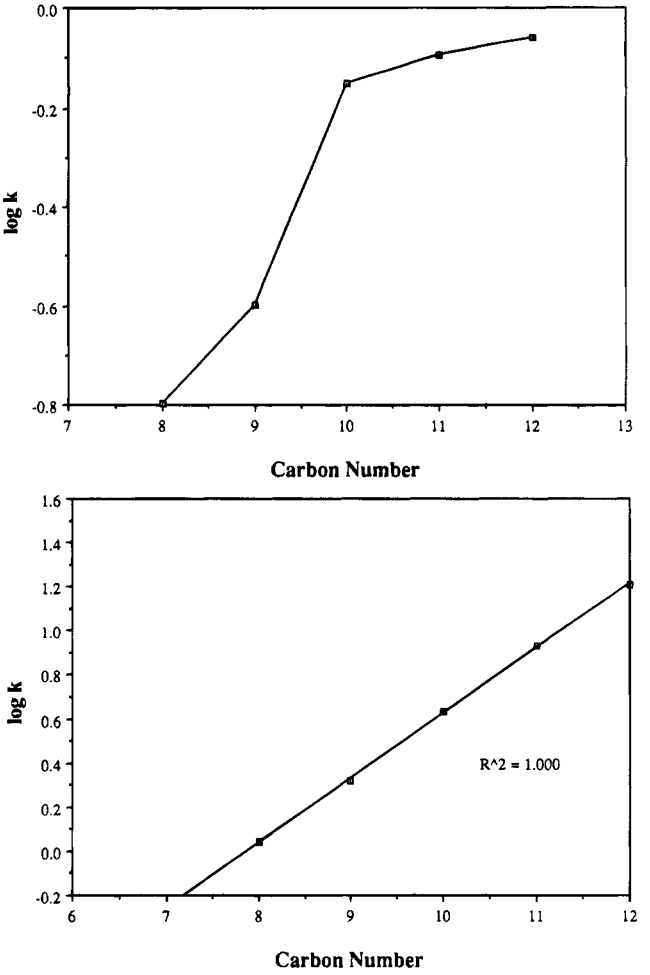

Figure 7. log k' vs carbon number (N_C) for *n*-alkylphenones in Brij 35/SDS. Data were obtained from Figure 1 (top) and Figure 6 (bottom).

the conditions of this study. Of course, the conditions for an infinite elution range with a given micellar system will vary depending on operating conditions, but the specific micellar composition for infinite elution can be determined by our approach (see Experimental Section, Table 1, Table 2, and Figures 1, 3, and 6) in a fairly short period of time with a modest amount of solution preparation.

In order to check the reproducibility of our method, infinite elution range conditions were obtained for a new surface-silanated capillary at pH 7.0. The capillary length was shortened to 30 cm (injection to detection). Table 2 lists the electroosmotic and micellar electrophoretic velocities obtained with this capillary. Once again, an infinite elution range was observed at approximately 0.012 M Brij 35/0.020 M SDS (see Figure 8). It should also be noted that both infinite elution range conditions were obtained in different laboratories and with different instruments and analysts.

The effect of increasing the SDS concentration from 0.020 to 0.040 M was also evaluated. We chose to investigate the effect of a greater SDS concentration with the untreated fused-silica capillary at a pH of 6.2. The use of 0.040 M SDS as opposed to 0.020 M SDS does not represent any advantages from the standpoint of higher operating currents and longer analysis times. Although the increase in operating currents is easily understood, explanation of the longer analysis times is a little more complicated. A higher concentration of SDS present in the running

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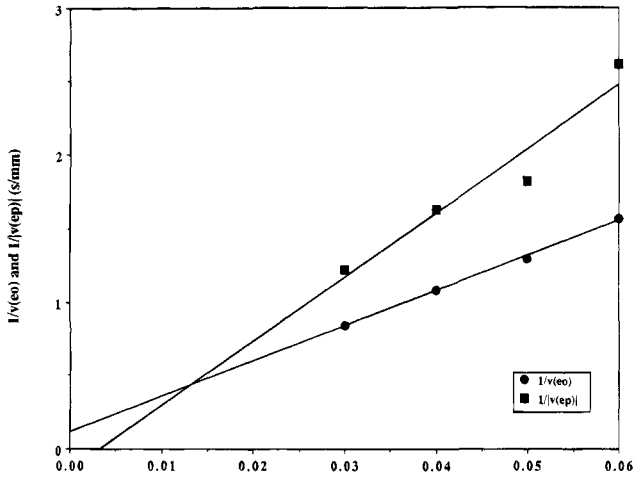

Figure 8. Dependence of the electroosmotic velocity and the electrophoretic velocity of the Brij 35/SDS mixed micelle on [Brij 35] for the surface-silanated fused-silica capillary at pH 7.0. Operating conditions are given in the Experimental Section.

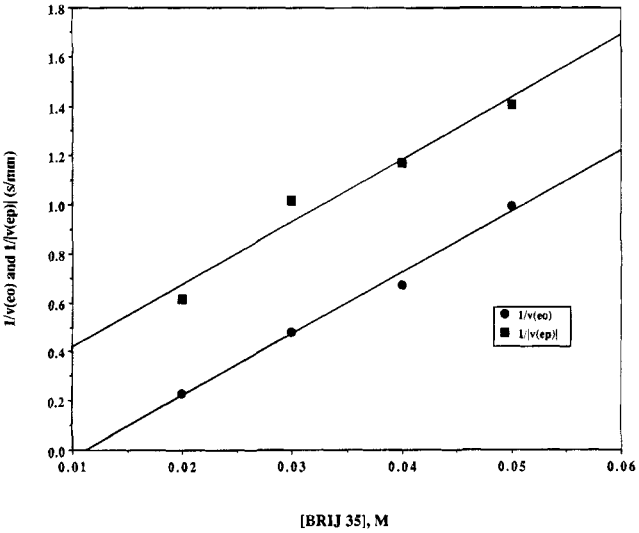

Figure 9. Dependence of the electroosmotic velocity and the electrophoretic velocity of the Brij 35/SDS mixed micelle on [Brij 35] for the untreated fused-silica capillary at pH 6.2. Micelle medium was [Brij 35]/0.040 M SDS in 10 mM phosphate buffer. Operating conditions are given in the Experimental Section.

buffer would mean a larger charge density on the surface of the mixed micelle and consequently much larger micellar electrophoretic velocities. Since the micellar electrophoretic velocity opposes the electroosmotic flow, which is reduced by the addition of a higher concentration of SDS, analysis times will be longer. With this in mind, the concentration of Brij 35 necessary to obtain an infinite elution range should be lower since both the electroosmotic and absolute micellar electrophoretic velocities will be closer in magnitude to begin with. Figure 9 shows the reciprocal electroosmotic and micellar electrophoretic velocities with the [Brij 35]/0.040 M SDS system. No convergence of electroosmotic and micellar electrophoretic velocities was observed with the 0.040 M SDS system at pH 6.2. In order to obtain an infinite elution range, now the buffer pH would have to be reduced even further, a clear disadvantage. We did not see any real advantage to operating with a higher SDS concentration; nevertheless, the

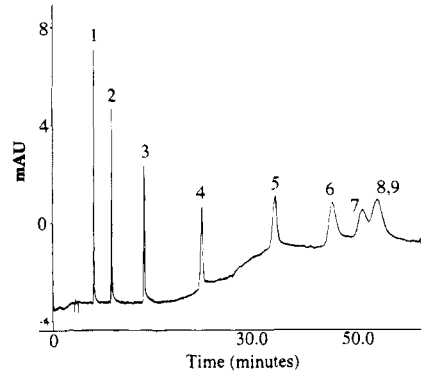

Figure 10. Separation of alkylphenone homologues using the untreated fused-silica capillary at pH 6.2 with a Brij 35 concentration of 0.014 M and an SDS concentration of 0.020 M.

results could prove useful in situations where a greater phase ratio is desired.

It is obvious from Figures 3 and 6 that operation at the Brij 35 concentration necessary to produce the infinite elution range leads to incredibly long analysis times, especially if very hydrophobic solutes are present in the sample. We therefore recommend using a Brij 35 concentration slightly higher than that obtained from the plot of reciprocal electroosmotic and micellar electrophoretic velocities. Figure 10 shows the separation of alkylphenone homologues using the untreated fused-silica capillary at pH 6.2 with a Brij 35 concentration of 0.014 M and an SDS concentration of 0.020 M. The elution range ($t_{mc}/t_o > 16$) is much larger, and, as a result of this, baseline resolution of two more homologues is possible in comparison to the separation obtained in Figure 1 using the conventional MEKC approach. In addition, partial resolution of an additional alkylphenone homologue was possible. It may be possible to obtain resolution of all the alkylphenone homologues using the zone-sharpening technique developed by Nielsen et al.[20,21] in combination with the infinite elution range demonstrated here. The use of field-amplified zone-sharpening under infinite elution range conditions could prove to be very valuable for MEKC. Zone-sharpening is very effective for the most hydrophobic solutes, which under infinite elution range conditions experience the greatest band-broadening, hence the two techniques coupled together could considerably improve separations obtained with MEKC.

It should be possible to achieve infinite elution ranges in MEKC with other types of mixed micelles or even through variation of the surfactant counterion. In a recent study, several tetraalkylammonium counterions were examined for their effects on selectivity and resolution in MEKC.[22] From the data reported in the investigation, it should be possible to achieve near-infinite elution range conditions using untreated fused-silica capillaries at neutral pH with the tetramethylammonium counterion.[22] It may also be possible to use micellar systems that do not interact with the wall of the capillary as much as the Brij 35 surfactant does. Clearly, further investigation is needed into the establishment of infinite elution ranges using different types of micellar systems. The tabulation of a broad range of micellar concentrations that yield infinite or near-infinite elution ranges ($100 < t_{mc}/t_o < 10\,000$)

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under conditions of relatively high electroosmotic flow (pH ≥ 6.5) should facilitate the optimization of the MEKC separations.[23-25]

CONCLUSIONS

Nonionic/anionic surfactant mixtures have been used rather sparingly in MEKC to date. However, the possibility of an infinite elution range through the use of mixed micellar systems could revolutionize MEKC by making this technique an attractive high-efficiency alternative to conventional reversed-phase LC. Although our results are preliminary, they clearly indicate that the elution range in MEKC can be increased greatly without painstaking adjustment of solution pH or direct modification of the capillary surface. In cases where the pH must be kept ≥7.0, it is recommended that the surface-silanated capillaries are used with at least a 10-min purge of phosphate buffer between runs to reduce the buildup of Brij 35 on the capillary surface. The use of untreated fused-silica capillaries (pH 6.2) is strongly encouraged, as the adsorption of Brij 35 on the capillary surface was much less than that with the silanated capillaries, which in turn leads to higher column efficiencies. It has also been demonstrated that this particular mixed micellar system exhibits retention characteristics similar to conventional reversed-phase LC via the linear relationship between log k' and carbon number of a homologous series. The approach described here for extending the elution range in MEKC is potentially of enormous value from both a theoretical and a practical standpoint.

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