

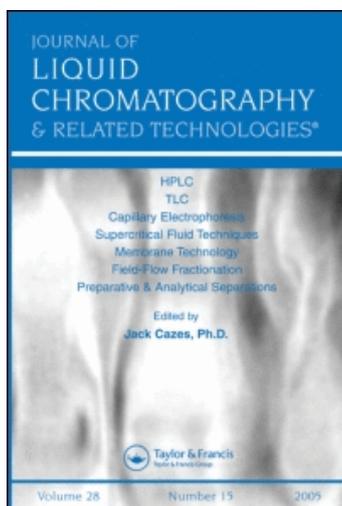
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### Retention Behavior of Highly Hydrophobic, Structurally Related Corticosteroids with SDS Based Microemulsion

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## Retention Behavior of Highly Hydrophobic, Structurally Related Corticosteroids with SDS Based Microemulsion

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**Abstract:** The present work describes the retention behavior of structurally related corticosteroids separated with SDS based microemulsion. The analytes include cortisone acetate (CA), hydrocortisone (H), hydrocortisone acetate (HA), prednisolone (P), prednisolone acetate (PA), and prednisone (Ps), which are not easily separated by the conventional MEKC. Surfactants with linear and planar structures have been compared for the separation of these analytes. By fine tuning the parameters, the results revealed that complete baseline separation could be achieved with a microemulsion consisted of 0.8% (w/w) *n*-octane, 3.6% (w/w) SDS, 6.6% (w/w) 1-butanol, and 89% (w/w) phosphate buffer (40 mM, pH 8.0), under an applied voltage of +7 kV and a column dimension of 53 cm (effective length, 30 cm) × 75 μm id. Although the composition of the microemulsion has a high recognition property for these structurally related compounds, the high affinity of these analytes with the pseudostationary phase made the analysis time very long. The effects of the addition of acetonitrile, methanol, and isopropanol have also been studied. Acetonitrile was found to have the greatest impact on this separation.

**Keywords:** Corticosteroids, EKC, Microemulsion, SDS

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## INTRODUCTION

In the past two decades, electrokinetic chromatography (EKC) has been regarded mainly as a special form of capillary electrophoresis. It has matured into a powerful analytical technique for the separation of uncharged analytes or analytes with matching ionic radii/charge ratios.<sup>[1-3]</sup> Capillary electrochromatography (CEC) has a true stationary phase.<sup>[4]</sup> Compared with CEC, the advantage of EKC is that the separation carrier replaces the stationary phase. Moreover, this pseudostationary phase is not immobilized, making EKC a more practical technique. Microemulsion electrokinetic chromatography (MEEKC) is an extension of the micellar electrokinetic chromatography (MEKC) principle, which was first presented by Watarai<sup>[5]</sup> in 1991. The separations are typically achieved using microemulsions, which are transparent solutions consisting of nanometer sized droplets dispersed throughout another immiscible liquid. MEEKC has been shown to exhibit superior separation efficiency compared with MEKC, due to improved mass transfer between the microemulsion droplets and the external aqueous phase mediated by the co-surfactant solvent.<sup>[6,7]</sup>

Several review articles have described the theory<sup>[6,8-10]</sup> and applications<sup>[2,7,10-12]</sup> of MEEKC. The type and concentration of surfactant significantly affects separations achieved in MEEKC by altering the droplet size and charge. SDS is one of the most useful surfactants employed as the pseudostationary phase in MEKC and MEEKC.<sup>[3,13]</sup> However, separations of some hydrophobic compounds using only SDS may be unsuccessful. There are only a few studies concerning MEEKC separation of steroids.<sup>[14-20]</sup> The use of anionic bile salts in place of SDS has been shown to offer different selectivity. Lucangioli et al.<sup>[14]</sup> reported that the microemulsion formed by phosphatidyl choline and isopropyl myristate were apparently better models to estimate the hydrophobicity of the betamethasone series. Pomponio et al.<sup>[15]</sup> reported that sodium taurodeoxycholate (STDC) showed better separation of corticosteroids than conventional SDS. However, a mix of surfactants (Brij 76 and STDC) and cyclodextrin were found to be essential to obtain adequate resolution. Wu et al.<sup>[20]</sup> reported the successful MEEKC separation of corticosteroids with diethyl L-tartrate as the oil phase.

Although bile salts, sodium cholate, or its derivatives exhibit greater discrimination ability for separating lipophilic compounds, they are more expensive than SDS. Moreover, we found that the strong reducing properties of the bile salts often result in the formation of deposits on the electrode surface after repeated experiments. This led to poor reproducibility of results. Therefore, SDS based

microemulsions were pursued for estimation of the feasibility for the separation of highly hydrophobic, structurally correlated corticosteroids. Parameters that affect the separation, such as the nature of the surfactant and cosurfactant, as well as pH and concentration of buffer were investigated. Furthermore, the influence of organic modifier on the retention of these analytes was also examined.

## EXPERIMENTAL

### Chemicals

All chemicals were analytical grade reagents from Merck (Darmstadt, Germany), unless otherwise stated. Purified water (18 M $\Omega$  cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions. *n*-Octane, 1-pentanol, isopropanol, cyclohexanol, sodium dihydrogen phosphate (Acros, Geel, Belgium), 1-butanol (Jassen, Beerse, Belgium), disodium hydrogen phosphate, trisodium phosphate (Merck, Darmstadt, Germany), benzyl alcohol, and methanol (Mallinckrodt, Saint Louis, MO, USA), dodecylbenzene, cortisone acetate (CA), hydrocortisone (H), hydrocortisone acetate (HA), prednisolone (P), prednisone (Ps), and prednisolone acetate (PA) (Sigma, Saint Louis, MO, USA), phosphoric acid, acetonitrile, cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and sodium deoxycholate (SDC) (Wako, Osaka, Japan) were purchased as indicated.

All solvents and solutions for MEEKC analysis were filtered through a 0.45- $\mu$ m PTFE (Millipore) or cellulose acetate (Whatman, Middlesex, UK) membrane.

### Instrumentation

All experiments were carried out in a laboratory built unit consisting of a UV-Vis detector (model UV-2075, Jasco, Japan) and a  $\pm$ 30 kV high voltage power supply (Gamma High Voltage Research Inc., Ormond Beach, FL, USA). For increasing the detection sensitivity, a capillary tube flow cell was used.<sup>[20]</sup> It was made by removing 10 mm of the polyimide coating and then bending the capillary into an inverse U shape. Electropherograms were recorded and processed with SISC-LAB (32) Ver. 2.01 (Taipei, Taiwan), running on the Windows XP operating system. Separations were carried out in fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75  $\mu$ m id and an effective length of 30 cm.

### Sample Preparation

Stock solutions ( $2 \text{ mg mL}^{-1}$ ) for each test compound were prepared in methanol. Each solution was then diluted with the microemulsion and stirred ultrasonically for 5 min before injection.

### Microemulsion Preparation

Microemulsions were prepared by weighing the desired quantities of surfactant and buffer. The cosurfactant and oil were added next, followed by sonication until the solution became transparent with no visible droplets on the surface.

### MEEKC Conditions

Before analysis, the columns were preconditioned with the running buffer. They were rinsed with methanol, NaOH (0.1 M) and pure water between runs at 5 min intervals. The sample was injected by hydrostatic mode (9 cm, 5 s). A detection wavelength of 254 nm was used for all samples.

## RESULTS AND DISCUSSION

The chemical structures of the model compounds are shown in Figure 1, where  $\log P_{ow}^{[21]}$  is the logarithm of the distribution coefficient between 1-octanol and water. Gabel-Jensen et al.<sup>[16]</sup> stated that the natures of the surfactant and cosurfactant are the most important factors in controlling the analytes migration order. The surfactant affects the droplet size and surface charge of the microemulsion, as well as the direction and quantity of the electroosmotic flow (EOF). Hence, the choice of surfactants (Figure 2) was considered first.

### Type of Surfactant

Initially, a microemulsion consisting of 0.8% (w/w) *n*-octane, 3.3% (w/w) SDS, 6.6% (w/w) *n*-butanol, and 89.3% (w/w) 50 mM phosphate buffer at pH 2.5, as that reported by Pomponio et al.<sup>[15]</sup> was used for this study. Under these conditions, the EOF is greatly suppressed and the neutral analytes were subjected to a chromatographic type separation. Similar elution order was obtained as in the cited paper.<sup>[15]</sup> The most

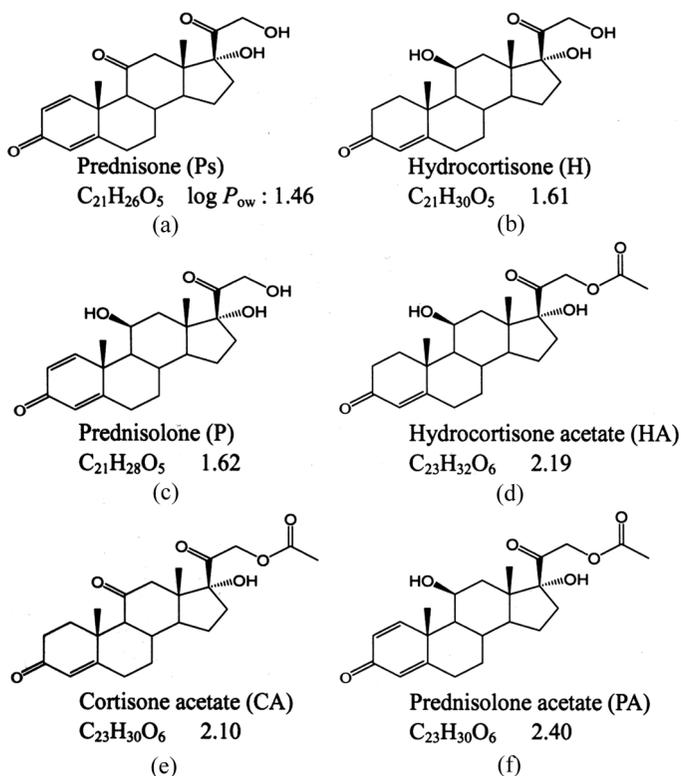


Figure 1. Chemical structures of the model compounds.

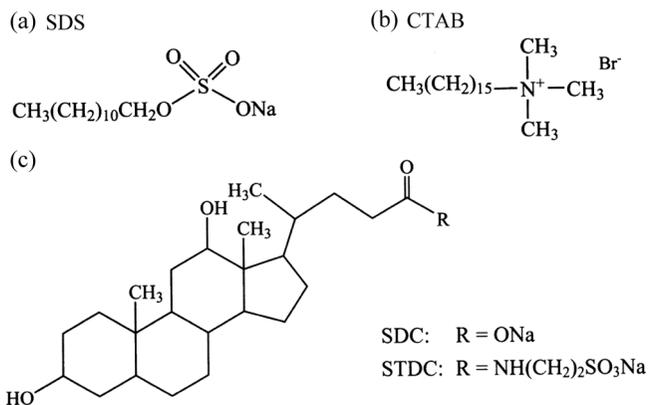
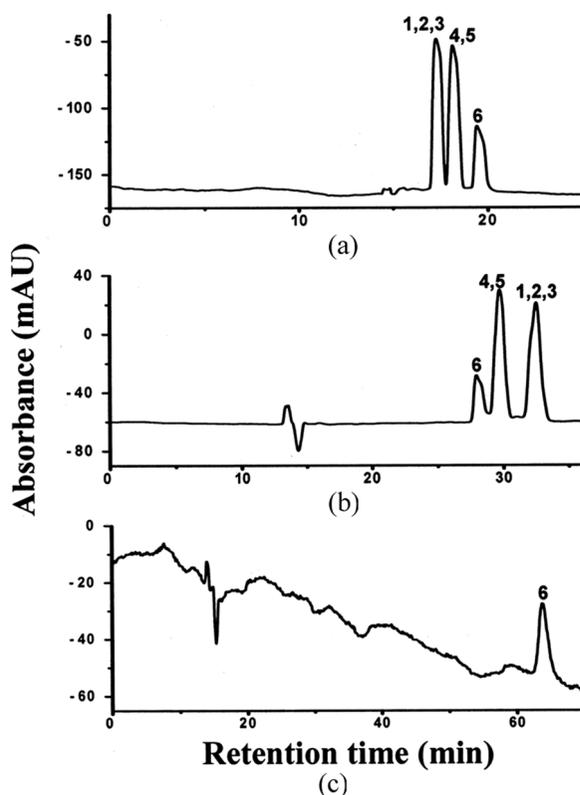


Figure 2. Chemical structures of the tested surfactants.

hydrophobic analytes with ester functional groups, PA, HA, and CA coeluted first, followed by the pair H and P. The least hydrophobic compound, Ps, eluted last (Figure 3a). Their shorter analysis time was mainly attributed to shorter column dimension. The secondary factors might be due to higher temperature and less hydrophobic oil phase.

For increasing the selectivity, Pomponio et al.<sup>[15]</sup> eventually used the mixed surfactant conditions (4.0% STDC, 2.5% Brij 76, 6.6% *n*-butanol, 1.36% *n*-heptane, and 85.54% 50 mM phosphate buffer pH 2.5) with the additive of  $\beta$ -cyclodextrin for complete separation. SDC has properties similar to those of STDC in the separation of xanthines.<sup>[22]</sup> Here, we tried



**Figure 3.** Electropherograms obtained by using microemulsion systems with different surfactants. Column: fused-silica capillary; 73 cm (30 cm)  $\times$  75  $\mu$ m id; sample injection: hydrostatic (9 cm, 5 s); sample concentration: 66.7  $\mu$ g mL<sup>-1</sup>; applied voltage: (a) and (c) -10 kV, (b) +10 kV; detection: 254 nm; mobile phase: 0.8% (w/w) *n*-octane, 3.3% (w/w) surfactant, 6.6% (w/w) 1-butanol, 89.3% (w/w) phosphate buffer (50 mM), and (a) SDS, pH 2.5 (b) SDC, pH 8.0 (c) CTAB, pH 2.5. Peak identification: 1. PA, 2. HA, 3. CA, 4. P, 5. H, 6. Ps.

using SDC to replace SDS in the microemulsion as that shown in Figure 3a. It was difficult to obtain a stable microemulsion unless the pH was raised to 8.0. SDC has a carboxylate residue. The event was attributed to less solubility of its molecular form in acidic solution. Compared with the microemulsion containing SDS in the suppressed EOF, the reverse retention order and longer retention time were indicated (Figure 3b). These phenomena can be explained by the fact that in MEEKC with suppressed EOF, neutral analytes with less retention time indicate greater retention factor or strongly partitioning of analytes into the microemulsion phase. The property is contrary to that of analytes with high EOF. Under alkaline conditions, the presence of a substantial EOF drives the negatively charged oil droplets toward the cathode. More hydrophobic solutes were strongly retained by the anionic pseudostationary phase. This made moving toward the detector end slower than for the less hydrophobic analytes. Corticosteroids possess a structure potentially capable of interacting with SDC. This allowed for greater solubilization of analytes in the microemulsion composed of SDC. Moreover, we found some deposits on the electrode surface after long-term use.

The performance of a cationic surfactant was also tested. A reversal in EOF was demonstrated due to bilayer formation. As the oil droplets of the microemulsion carry a number of positive charged CTAB, they migrate toward the cathode opposite to the EOF. We changed the polarity for the sample injection. Thus, the more lipophilic substances exhibit longer migration time. In this case, we observed only one peak (Ps) within 70 min (Figure 3c), which can be explained by the fact that the EOF was greatly reduced.

### Type of Cosurfactant

In an attempt to get better selectivity of the results shown in Figure 3a, the 1-butanol cosurfactant was replaced with 1-pentanol, 1-hexanol, and cyclohexanol. Turbidity was observed with 1-hexanol as the cosurfactant, which might reflect an inappropriate ratio. The elution profiles for the other three cosurfactants were the same. The more hydrophobic CA, HA, and PA eluted first. The pair H and P was next, and Ps was the last to elute. However, a longer elution time was required for 1-pentanol. A substantially shorter retention time and poor reproducibility were demonstrated with cyclohexanol. The results indicated that the solubilization of these solvents into the oil droplet decreases in the order: 1-pentanol > 1-butanol > cyclohexanol. The molecular shape of cyclohexanol does not seem to favor the formation of a stable oil in water microemulsion. This resulted in less interaction with the analytes. Thus, 1-butanol was preferred as the cosurfactant.

### pH of the Aqueous Solution

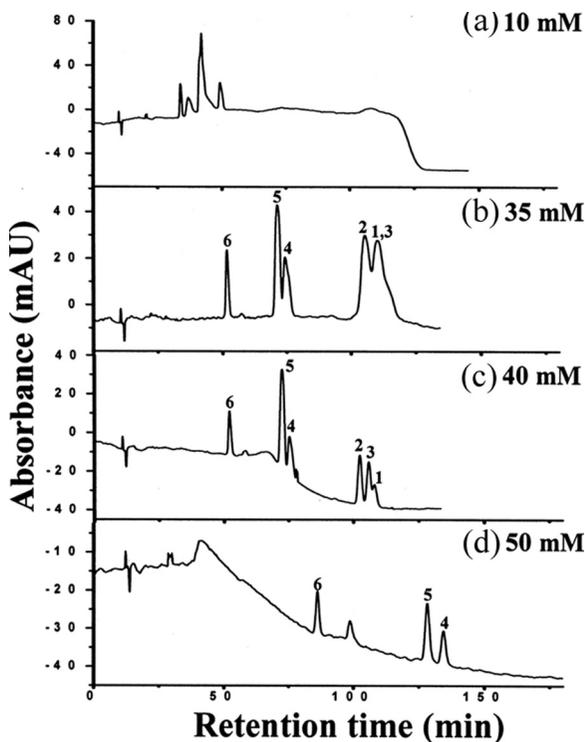
For an improvement of the separation, the pH was increased to 3.5–4.5. However, only a single peak for the neutral marker, methanol, was observed. As the pH was increased to 8.0, Ps and partial resolution for H and P were observed. By further increasing the pH to 9.0, faster EOF made the pair of H and P coelute. Hence, pH 8.0 was chosen for further experiments.

### Buffer Concentration

The effects of phosphate buffer concentration (10 to 50 mM) on separation were studied. The concentration variation had a pronounced effect, with substantial differences in retention time and peak resolution (Figure 4). A strong EOF at 10 mM phosphate buffer made the analytes less retentive in the oil droplet of the microemulsion. Four peaks were found when we injected six analytes (Figure 4a). Here, we did not assign the peaks. Better resolution was observed as the concentration was increased beyond 35 mM. The reason for this, likely results from a reduced EOF, due to the changes in ionic strength and zeta potential that occurred as the phosphate concentration was increased. This made the retention time longer, especially for the more lipophilic compounds (CA, HA, and PA). Considering the analysis time, 40 mM was chosen as the optimum concentration at which the retention affinity was  $Ps < H < P < HA < CA < PA$  (Figure 4c).

### Concentration of the Surfactant

Figure 5 shows the effect of SDS concentration in the microemulsion buffer on retention time. At an SDS concentration less than 2.8% (w/w), poor resolution was observed. In these cases, Ps was eluted the earliest, P and H were partially resolved, and the last peak contained PA, HA, and CA. An increase in SDS concentration resulted in longer analysis time due to the greater retention factor, which was in turn caused by an increase in phase ratio. Six peaks for the analyte mixture can be found at a concentration of 3.3%, although PA and CA are still not completely separated (Figure 5a). With a further increase in SDS concentration, selectivity was significantly increased (Figure 6). However, an increase in SDS concentration to 3.8% led to poor resolution, especially for the more hydrophobic substances. This might be due to Joule heating leading to an increase in the plate height. The greater interaction of the oil droplet with the analytes made discrimination more difficult. Separation of the pairs of H and P, as well as CA and PA, became poor.

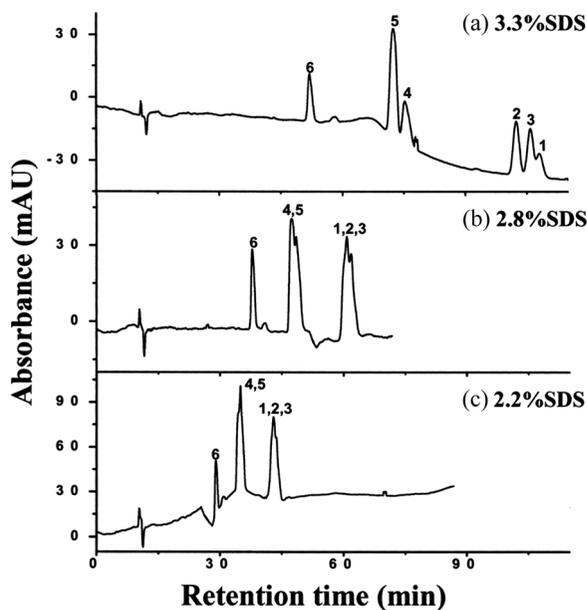


**Figure 4.** Electropherograms of corticosteroids separated at different phosphate buffer concentrations. Conditions were as in Figure 3, except the mobile phase: 0.8% (w/w) *n*-octane, 3.3% (w/w) SDS, 6.6% (w/w) 1-butanol, 89.3% (w/w) phosphate buffer (pH 8.0) at (a) 10 mM (b) 35 mM (c) 40 mM (d) 50 mM and applied voltage: +10 kV. Peak identification: 1. PA, 2. HA, 3. CA, 4. P, 5. H, 6. Ps.

The best separation was achieved at 3.6% SDS. The retention order was increased as  $\text{Ps} < \text{H} < \text{P} < \text{HA} < \text{CA} < \text{PA}$ , which almost corresponded to the hydrophobic character of the analytes, except for the pair CA and HA. CA has a carbonyl group on  $\text{C}_{11}$ , whereas HA is an alcohol. Stronger hydrogen bond formation between HA and the pseudostationary phase explains the unexpected retention order of these two analytes. Separation efficiencies at the above specified conditions were shown as Table 1.

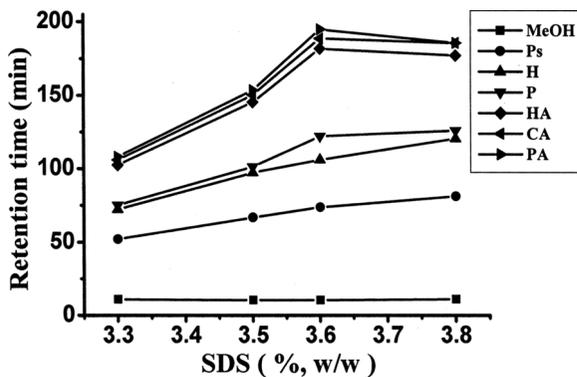
### Effect of Field Strength

Octane has a long alkyl chain. As a result of the highly lipophilic nature of the model corticosteroid compounds, slower migration was observed



**Figure 5.** Electropherograms of corticosteroids at different concentrations of SDS. Conditions as in Figure 4, except that the mobile phase was 0.8% (w/w) *n*-octane, 6.6% (w/w) 1-butanol, 89.3–90.4% (w/w) phosphate buffer (40 mM, pH 8.0). Peak identification: 1. PA, 2. HA, 3. CA, 4. P, 5. H, 6. Ps.

due to strong nonpolar-nonpolar interactions with the oil droplet. High voltages were applied to speed up the migration but excessive Joule heating made the resolution poor. By varying the total column length and



**Figure 6.** Effect of SDS concentration on the separation of corticosteroids. Conditions as in Figure 4, except that the mobile phase was 0.8% (w/w) *n*-octane, 6.6% (w/w) 1-butanol, 88.8–89.3% (w/w) phosphate buffer (40 mM, pH 8.0).

**Table 1.** MEEKC separation of corticosteroids at different SDS concentration<sup>a</sup>

Analyte	3.3% (w/w)			3.6% (w/w)			3.8% (w/w)		
	k <sup>b</sup>	N(m <sup>-1</sup> ) <sup>c</sup>	R <sub>s</sub> <sup>d</sup>	k	N(m <sup>-1</sup> )	R <sub>s</sub>	k	N(m <sup>-1</sup> )	R <sub>s</sub>
Ps	3.8	4900	—	6.1	1600	—	6.4	23300	—
H	5.7	9200	5.79	9.2	7800	4.54	10.0	9700	9.55
P	6.0	10400	0.83	10.8	5600	2.42	10.5	7400	0.87
HA	8.5	16000	7.48	16.5	14200	8.04	15.2	10600	6.83
CA	8.8	28500	1.02	17.2	20900	1.04	16.0	7100	0.92
PA	9.0	43500	0.81	17.8	23800	1.02	16.0	7100	0.00

<sup>a</sup>Fused-silica capillary: 73 cm (30 cm) × 75 μm id; microemulsion: 0.8% (w/w) *n*-octane, 6.6% (w/w) 1-butanol, 88.8–89.3% (w/w) phosphate buffer (40 mM, pH 8.0); sample injection: hydrostatic (9 cm, 5 s); sample concentration: 66.7 μg mL<sup>-1</sup>; applied voltage: +10 kV; detection: 254 nm.

<sup>b</sup>Capacity factor,  $k = (t_r/t_o - 1)/(1 + t_r/t_{mc})$ , where  $t_r$ ,  $t_o$  (with methanol as marker) and  $t_{mc}$  (with dodecylbenzene as tracer) are the retention times of the solute, the void volume due to EOF, and the microemulsion, respectively.

<sup>c</sup>Theoretical plate,  $N = 5.54 (t_r/w_{1/2})^2$ ,  $w_{1/2}$  is the peak width at half height.

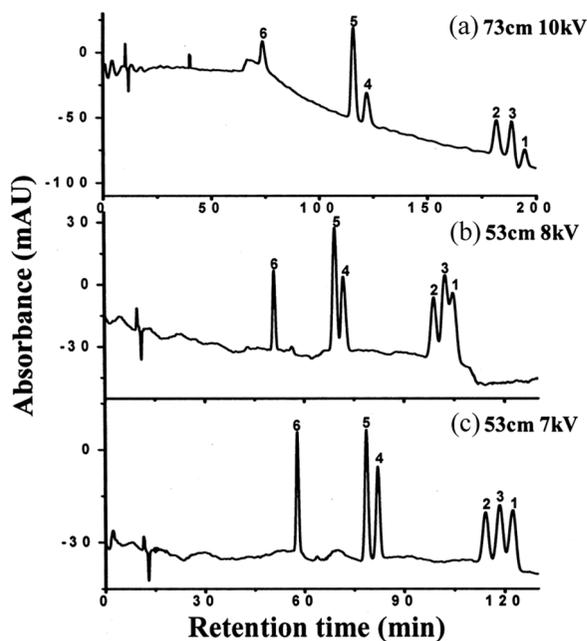
<sup>d</sup>Resolution,  $R_s = 2 (t_{r2} - t_{r1})/(w_2 + w_1)$ .

the applied voltage, it was found that complete baseline separation of the six corticosteroids was obtained with a shorter column (53 cm total length) and a lower applied voltage of +7 kV (Figure 7c).

## Organic Modifiers

Organic solvents are usually added to the buffer to improve the separation.<sup>[9,23]</sup> In a subsequent experiment, the addition of methanol, acetonitrile, and isopropanol was tested. Increasing the amount of methanol led to lower conductivity, slower EOF, and reduced polarity of the aqueous phase. This resulted in a higher distribution of the analyte in the aqueous phase, which reduced the interaction force of the analytes with the microemulsion. Hence, faster elution was observed (Table 2). When the concentration of methanol was further increased to 15%, coelution of HA and CA was indicated.

In an attempt to enhance the separation of the HA/CA pair, acetonitrile was substituted for methanol (Table 3). The greater zeta potential of the capillary due to the higher dielectric constant and lower viscosity of the acetonitrile-water mixture compared to the methanol-water mixture, gives a relatively higher EOF. This led to lower retention of the analyte in the oil droplet of the microemulsion, and stronger hydrogen bonding with the aqueous buffer (Figure 8). We found that the analysis time



**Figure 7.** Electropherograms of corticosteroids separated with different capillary lengths and applied voltages. Conditions were as in Figure 4, but the column dimension (applied voltage) (a) 73 cm (+10 kV), (b) 53 cm (+8 kV), (c) 53 cm (+7 kV) (effective length, 30 cm)  $\times$  75  $\mu$ m id; and mobile phase: 0.8% (w/w) *n*-octane, 3.6% (w/w) SDS, 6.6% (w/w) 1-butanol, 89% (w/w) phosphate buffer (40 mM, pH 8.0). Peak identification: 1. PA, 2. HA, 3. CA, 4. P, 5. H, 6. Ps.

**Table 2.** Capacity factor for the MEEKC separation of corticosteroids using different concentrations of methanol<sup>a</sup>

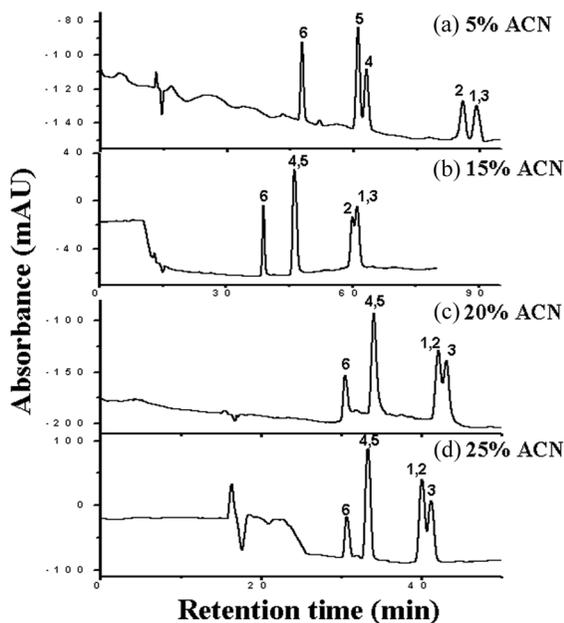
Analyte	4%	8%	15%
Ps	4.19	3.65	1.89
H	6.17	5.37	2.70
P	6.46	5.66	2.81
HA	9.29	7.87	3.75
CA	9.56	8.05	3.75
PA	9.93	8.42	3.93

<sup>a</sup>Conditions were as in Table 1, except the fused-silica capillary: 53 cm (30 cm)  $\times$  75  $\mu$ m id; microemulsion: 0.8% (w/w) *n*-octane, 3.6% (w/w) SDS, 6.6% (w/w) 1-butanol, 74–85% (w/w) phosphate buffer (40 mM, pH 8.0) and the applied voltage: +7 kV.

**Table 3.** Capacity factor for the MEEKC separation of corticosteroids using different concentrations of acetonitrile<sup>a</sup>

Analyte	0%	5%	15%	20%	25%
Ps	4.07	2.61	1.96	0.97	0.88
H	5.90	3.61	2.52	1.21	1.04
P	6.20	3.76	2.52	1.21	1.04
HA	9.02	5.48	3.56	1.73	1.45
CA	9.39	5.72	3.64	1.79	1.52
PA	9.74	5.72	3.64	1.73	1.45

<sup>a</sup>Conditions were as in Table 1, except the fused-silica capillary: 53 cm (30 cm) × 75 μm id; microemulsion: 0.8% (w/w) *n*-octane, 3.6% (w/w) SDS, 6.6% (w/w) 1-butanol, 64–89% (w/w) phosphate buffer (40 mM, pH 8.0) and applied voltage: +7 kV.



**Figure 8.** Electropherograms of corticosteroids using different concentration of acetonitrile. Column: fused-silica capillary; 53 cm (30 cm) × 75 μm id; sample injection: hydrostatic (9 cm, 5 s); sample concentration: 66.7 μg mL<sup>-1</sup>; applied voltage: +7 kV; detection: 254 nm; mobile phase: 0.8% (w/w) *n*-octane, 3.6% (w/w) surfactant, 6.6% (w/w) 1-butanol, 64–84% (w/w) phosphate buffer (40 mM, pH 8.0), ACN, x (w/w). Peak identification: 1. PA, 2. HA, 3. CA, 4. P, 5. H, 6. Ps.

was reduced to 90 min even with the addition of only 5% acetonitrile. The faster analysis, however, resulted in overlapping of PA and CA. As the concentration of acetonitrile was further increased to 15%, not only did the pair P/H and CA/PA elute simultaneously, but there was also some overlap between HA and the CA/PA pair. When the concentration of acetonitrile increased to  $\geq 20\%$ , peak height increased due to greater solubility of the analyte. Weak retention in the oil droplet, however, made PA coelute with HA.

Isopropanol is more hydrophobic than other cosolvents and can act as a cosurfactant.<sup>[23]</sup> With isopropanol in the aqueous phase of the microemulsion, the solubility of the corticosteroids was usually higher and their affinity for the oil droplet phase was reduced. The addition of only 4% isopropanol significantly reduced analysis time. Due to the rather weak interactions between the corticosteroids and the microemulsion, poorer resolution than that with methanol or acetonitrile was exhibited.

## CONCLUSIONS

The ability to make changes in resolution and to control selectivity is one of the most important concepts in separation science. Although we have made good MEEKC separation of ten corticosteroids with diethyl-L-tartrate as oil phase,<sup>[20]</sup> complete separation of six corticosteroids, H, P, Ps, HA, CA, and PA could be achieved by only the typical SDS based microemulsion in this work. The microemulsion consisted of 0.8% (w/w) *n*-octane, 3.6% (w/w) SDS, 6.6% (w/w) 1-butanol, and 89% (w/w) phosphate buffer (40 mM, pH 8.0). Comparative studies of the separations have been reported for these very similar structurally related corticosteroids.<sup>[15,18]</sup> However, no successful result was obtained from their works. Bile salts of planar structure have better recognition ability for the steroids. However, microemulsion with other additive and mix surfactants are necessary for the MEEKC separation of these compounds.<sup>[15]</sup>

Here, the corticosteroids could be highly recognized by SDS based microemulsion, although the analysis time was very long. The results indicate that the preparation of highly selective MEEKC stationary phases is a very easy and practical technique compared with CEC. Microemulsions are thermodynamically stable systems consisting of two immiscible liquids separated and stabilized by a monolayer of surfactant. By varying the composition of microemulsion using the phase diagram of the ternary system,<sup>[24]</sup> even the liquid crystal phase with shape selectivity can be prepared. We can conclude that microemulsions as pseudostationary phases of capillary electrophoresis are indeed vivid, versatile, and highly promising in the separation science.

## ABBREVIATIONS

MEKC, micellar EKC; MEEKC, microemulsion EKC; CA, cortisone acetate; H, hydrocortisone; HA, hydrocortisone acetate; Ps, prednisone; P, prednisolone; PA, prednisolone acetate; SDS, sodium dodecyl sulfate; SDC, sodium deoxycholate; STDC, sodium taurodeoxycholate; CTAB, cetyltrimethylammonium bromide.

## ACKNOWLEDGMENT

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