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Micellar electrokinetic chromatography employing sodium alkyl sulfates and Brij 35®[a]

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ABSTRACT

Sodium decyl and dodecyl sulfates were evaluated as micellar phases for the micellar electrokinetic chromatographic separation of ASTM test mixture LC-79-2. Selectivity was similar in each system but differed from the selectivity obtained in reversed-phase high-performance liquid chromatography. Despite separation efficiencies of approximately 150 000 theoretical plates per 50 cm, benzene and benzaldehyde coeluted in all concentrations of both surfactants employed. Separation was, however, readily achieved by addition of Brij 35® [polyoxyethylene(23)dodecanol] to the micellar phase.

INTRODUCTION

Micellar electrokinetic chromatography (MEKC), as introduced by Terabe and co-workers[1,2], is a method of microscale chemical separation based on the electrokinetic effects which occur when a buffer-filled fused-silica capillary is subjected to an electric field[3]. The electrokinetic phenomena cause two dissimilar phases (a charged micellar phase and an aqueous buffer) to migrate at different velocities. Specifically, the aqueous phase migrates at a velocity dictated by electroosmotic flow and the micellar phase at a velocity that is the vector sum of the electroosmotic flow and the micelle's electrophoretic mobility. Solutes are separated in such a system based on partitioning between each phase. Therefore, MEKC may be considered analogous to conventional chromatography; the only exception being that the conventional stationary phase is replaced by a micellar phase, which is itself mobile. As a result, modifications must be made to conventional definitions of chromatographic parameters. In MEKC the capacity factor, k', may be expressed as[1]

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \quad (1)$$

[a] Brij35® is a Registered Trademark of ICI Americas, Inc.

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where t_R is the retention time of an analyte, t_0 the retention time of a solute which distributes exclusively into the aqueous phase and t_{mc} the retention time of a solute which distributes exclusively into the micellar phase. Accordingly, the master resolution equation for MEKC becomes[2]

$$R_s = \frac{N^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 + k'_2}\right)\left[\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'_1}\right] \quad (2)$$

where R_s is the resolution, N the number of theoretical plates, k'_2 and k'_1 the capacity factors of solutes 2 and 1, respectively, and α the selectivity ($\alpha = k'_2/k'_1$).

By examining the master resolution equation, the mobility of the micellar phase is seen to be detrimental to resolution. However, since electroosmotic flow can be changed via changes in the capillary surface[4–6], buffer pH[7–9] and buffer concentration[10], this limitation may be overcome. The remaining parameters appear favorable in comparison to conventional high-performance liquid chromatography (HPLC). The flat flow profile created by electroosmotic flow[11] can provide for chemical separations with efficiencies in excess of 500 000 theoretical plates per m (ref. 12). Additionally, a vast number of micellar phases are available for optimization of selectivity.

To date most applications have employed sodium dodecyl sulfate (SDS) as the micellar phase[13–17]. However, the use of sodium decyl sulfate (STS), sodium tetradecyl sulfate, sodium dodecanesulfonate, dodecyltrimethylammonium chloride, cetyltrimethylammonium chloride and sodium *n*-dodecanoyl-L-valinate have also been briefly explored[2,18–20]. The addition of tetraalkyl ammmonium salts[21], methanol[22,23] and metal ions[24] to SDS micelles has also been shown to alter selectivity.

The purpose of the research described here is to further characterize SDS and STS micellar phases, and to compare the selectivity obtained in each system with the selectivity observed in reversed-phase HPLC. The influence of Brij 35® [polyoxyethylene(23)dodecanol] on selectivity is also briefly explored.

EXPERIMENTAL

Surfactant solutions of SDS and STS (Aldrich, Milwaukee, WI, U.S.A.) were prepared at the specified concentrations in 0.01 *M* disodium hydrogenphosphate, pH 7.00. To obtain data which can readily be compared with reversed-phase HPLC, ASTM test mixture LC-79-2 (refs. 25, 26), dissolved in each of the surfactant solutions under investigation, was used as the sample. This test mixture contains 1.5 mg/ml benzyl alcohol, 0.02 mg/ml benzaldehyde, 0.025 mg/ml acetophenone, 1.04 mg/ml benzene, 0.4 mg/ml methyl benzoate and 0.054 mg/ml dimethyl terephthalate. To calculate thermodynamic parameters, 1% (v/v) methanol and a small amount of Sudan III were added to the conventional test mix, to mark, respectively, the elution times of completely unretained and completely retained solutes (*i.e.*, t_0 and t_{mc})[2].

MEKC analyses were performed, at ambient temperature, in an 80 cm × 100 μm I.D. fused-silica capillary (Polymicro, Phoenix, AZ, U.S.A.) with a sample introduction–detector distance (effective length) of 50 cm. Separations were performed at +15 kV, as supplied by a Spellman Model RHR 30 high-voltage power supply (Spellman, Plainview, NY, U.S.A.) and monitored by means of an ISCO Model CV4

capillary electrophoresis absorbance detector (ISCO, Lincoln, NE, U.S.A.) set at 254 nm. Samples were introduced into the capillary by siphoning for 10 s at an elevation of 3.8 cm. This provides a sample volume of approximately 10 nl.

To evaluate the effect of Brij 35 on the separation of benzaldehyde and benzene a 0.025 *M* SDS–0.025 *M* Brij 35 (Fisher Scientific, Raleigh, NC, U.S.A.) solution was prepared as above. Benzene (0.84 mg/ml) and benzaldehyde (0.17 mg/ml) were analyzed as described, at a wavelength of 215 nm.

RESULTS AND DISCUSSION

The separation of ASTM test mixture LC-79-2 obtained in 0.05 *M* SDS is shown in Fig. 1. All components with the exception of benzene and benzaldehyde are shown to be adequately resolved. To resolve these coeluting components, optimization in accordance with the master resolution equation for MEKC (eqn. 2) was attempted. An approach similar to that applied in conventional HPLC was employed[27]; thus, initial efforts focused on changing the SDS concentration to effect a more favorable capacity factor for the separation. As seen from the coefficients of correlation in Table I, solute capacity factors are readily predicted as a function of SDS concentration. However, for the range of concentrations utilized (0.025–0.075 *M*), it was not possible to effect the separation of benzene and benzaldehyde.

This observation is in agreement with the master resolution equation. Making the assumption that $k'_1 = k'_2$, the optimum capacity factor for separation of the critical pair is given by the maximum of the function[2]

$$f(k') = \left(\frac{k'}{1 + k'}\right)\left[\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'}\right] \quad (3)$$

For a t_0/t_{mc} value of 0.291 $\pm$ 0.003, as observed for 0.05 *M* SDS, the maximum (0.3) occurs at a capacity factor of approximately 1.85. However, $f(k')$ is constant to within 1% from $k' = 1.6$–2.2; thus the capacity factor is essentially optimized in 0.075 *M* SDS ($k' = 1.63$). This maximum provides an enhancement in resolution by a factor of only 1.08 compared to 0.05 *M* SDS ($k' = 1.03$) and it is therefore not surprising that resolution is not effected by increasing the micellar concentration.

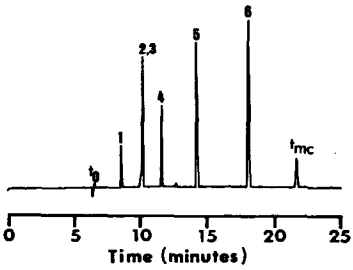

Fig. 1. MEKC separation of ASTM test mixture LC-79-2. Conditions: 80 cm × 100 μm I.D. fused-silica capillary (effective length 50 cm); 0.05 *M* SDS in 0.01 *M* Na_2HPO_4 (pH 7.00); +15 kV applied voltage; UV absorbance detection at 254 nm. Peaks: 1 = benzyl alcohol; 2,3 = benzene and benzaldehyde; 4 = acetophenone; 5 = methyl benzoate; 6 = dimethyl terephthalate.

TABLE I

SOLUTE CAPACITY FACTORS AND t_0/t_{mc} RATIOS *VS.* SDS CONCENTRATION

All values are based on four determinations.

Solute	*SDS concentration*			*r*
	0.025 M	*0.050 M*	*0.075 M*	
Benzyl alcohol	0.28 ± 0.01	0.55 ± 0.02	0.85 ± 0.01	0.99985
Benzene	0.54 ± 0.02	1.09 ± 0.02	1.63 ± 0.03	0.99998
Acetophenone	0.84 ± 0.02	1.71 ± 0.03	2.52 ± 0.03	0.99980
Methyl benzoate	1.67 ± 0.04	3.47 ± 0.05	5.16 ± 0.03	0.99984
DMT[a]	5.38 ± 0.08	10.7 ± 0.3	15.7 ± 0.5	0.99982
t_0/t_{mc}	0.350 ± 0.008	0.291 ± 0.003	0.292 ± 0.003	

[a] Dimethyl terephthalate.

Based on the results of Terabe *et al.*, who noted a decrease in t_0/t_{mc} with an increase in surfactant concentration, it may be argued that as surfactant concentration is increased from 0.05 to 0.075 *M*, an increase in the maximum of f(k') should result. Thus an enhancement in resolution greater than predicted above may be possible. In this work, the experimentally observed t_0/t_{mc} was 0.292 ± 0.003 in 0.075 *M* SDS, indicating that the optimum capacity factor is unaltered. However, in 0.025 *M* SDS the t_0/t_{mc} ratio did increase. By virtue of a decreased t_{mc} value the observed ratio was 0.350 ± 0.008. Reasons for these results are currently under investigation. Speculatively, the variations may be due to a combination of the numerous temperature-dependent parameters which can change as a result of Joule heating as voltage is applied to the capillary. The current drawn and therefore the Joule heat generated, increased with increasing SDS concentration. Under the conditions employed (80 cm × 100 μm capillary; 15 kV applied voltage), the currents drawn in the 0.025, 0.05 and 0.075 *M* SDS solutions were 90, 110 and 135 μA, respectively.

In light of the reduction in resolution caused by the mobility of the micellar phase, it is interesting to compare resolution in MEKC with the resolution obtainable in HPLC. Rewriting eqn. 2 as

$$R_s = \frac{N^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right) f(k') \tag{4}$$

and invoking that the same resolution is required of each separation procedure, it follows that

$$\frac{N_H^{1/2}}{4}\left(\frac{\alpha_H - 1}{\alpha_H}\right) f(k')_H = \frac{N_M^{1/2}}{4}\left(\frac{\alpha_M - 1}{\alpha_M}\right) f(k')_M \tag{5}$$

where the subscripts H and M denote HPLC and MEKC, respectively. When SDS was used as the micellar phase the maximum of $f(k')_M$ was 0.3 and the efficiency (N_M) obtained in a capillary with an effective length of 50 cm approximately 150 000 plates. Assuming that a similar separation can be achieved by HPLC at a capacity factor of 10,

TABLE II

SELECTIVITY (α) BETWEEN SELECTED SOLUTE PAIRS IN 0.05 *M* SDS AND 0.05 *M* STS

All values are based on four determinations.

Solute pair	*α in 0.05 M SDS*	*α in 0.05 M STS*
Benzyl alcohol–benzene	1.97 ± 0.03	1.92 ± 0.01
Benzene–acetophenone	1.569 ± 0.003	1.48 ± 0.02
Acetophenone–methyl benzoate	2.03 ± 0.02	1.91 ± 0.02
Methyl benzoate–DMT	3.09 ± 0.04	2.73 ± 0.04

$f(k')_H = 0.91$. Thus if selectivity is the same in each system (*i.e.*, $\alpha_H = \alpha_M$), the resolution obtained by MEKC is similar to the resolution provided by an HPLC column generating 16 000 theoretical plates.

To improve the resolution provided by MEKC, N_M may be increased by employing a capillary with a longer effective length, or by optimizing the operating parameters governing dispersion[28]. Alternatively, efforts may be made to improve selectivity or decrease the t_0/t_{mc} ratio. In an attempt to obtain these advantages, 0.05 *M* STS was used instead of SDS as the micellar phase. STS provides an extended elution range by virtue of increased electrophoretic mobility (t_0/t_{mc} of STS = 0.235 ± 0.006) and as a result of a shorter alkyl chain length may perhaps be expected to provide different selectivity than SDS. As experimentally observed, 0.05 *M* STS also failed to separate benzene and benzaldehyde. In fact, as shown in Table II, differences in selectivity between SDS and STS are minor. Similar results have been reported by Burton *et al.*[19].

Attempts to improve resolution by employing STS of different concentrations were not feasible. The sample was insoluble in 0.025 *M* STS and 0.075 *M* STS caused an appreciable decrease in the signal-to-noise ratio. The latter is attributed to the generation of Joule heat which is not effectively dissipated by the detector. The current drawn, for an applied potential of 15 kV, was 210 μA for the 0.075 *M* STS solution.

Additional studies showed that other sodium alkyl sulfates are not suitable for MEKC at ambient temperature. Sodium octyl sulfate at a concentration of 0.075 *M* did not dissolve the sample, presumably because this concentration is below the surfactant's critical micelle concentration[29]. Sodium tetradecyl sulfate was insoluble in the operating buffer at a concentration of 0.025 *M*. It should be noted, however, that the solubility increases markedly at elavated temperatures. As shown by Terabe *et al.*[2] 0.05 *M* sodium tetradecyl sulfate may be used at 35°C.

For ASTM test mixture LC-79-2, the selectivity obtained in SDS and STS does however differ from that obtained by conventional reversed-phase HPLC and by reversed-phase HPLC employing Brij 35 as the mobile phase[26]. The orders of elution are listed in Table III. Notably, benzene elutes at different relative times in each separation mode.

Since solvent–micelle partitioning is responsible for part of the selectivity mechanism in micellar chromatography and under the premise that the polar head group changes selectivity in MEKC[19], it is logical to explore the use of Brij 35 as a micellar phase for MEKC. However, because Brij 35 is non-ionic, it cannot migrate

TABLE III

ORDER OF ELUTION OF ASTM LC-79-2 SAMPLE COMPONENTS UNDER VARIOUS LC SEPARATION CONDITIONS

	From ref. 26	*From ref. 26*	*This work*
"Stationary phase":	RP-18	RP-18	SDS and STS
Mobile phase:	30:70 (v/v) acetonitrile–water	6% Brij 35	0.01 *M* Na_2HPO_4 pH 7.00
Elution order:	(1) benzyl alcohol (2) benzaldehyde (3) acetophenone (4) methyl benzoate (5) benzene (6) dimethyl terephthalate	(1) benzyl alcohol (2) benzaldehyde (3) acetophenone (4) benzene (5) methyl benzoate (6) dimethyl terephthalate	(1) benzyl alcohol (2, 3) benzaldehyde and benzene (4) acetophenone (5) methyl benzoate (6) dimethyl terephthalate

electrophoretically. Therefore it is of little use by itself in MEKC. However, the feasibility of adding Brij 35 to a charged micelle, forming charged mixed micelles, remains. The separation of benzene and benzaldehyde obtained in 0.025 *M* SDS–0.025 *M* Brij 35 is shown in Fig. 2. Benzene is retained longer than benzaldehyde, indicating that the nature of the surfactant's polar head group plays an important role in solute retention. It is unclear, however, specifically why selectivity is changed. Several different models for the solubilization of benzene in micelles have been proposed[30].

The specific attributes of SDS–Brij 35 mixed micelles as micellar phases for MEKC will be investigated in a subsequent paper. However, a major advantage of Brij 35, as opposed to charged additives, is that it may be added to the micellar phase without an increase in Joule heating. The current resulting from both the 0.025 *M* SDS and the 0.025 *M* SDS–0.025 *M* Brij 35 solutions was 90 μA. Additionally, as noted previously[26], Brij 35 has a high cloud-point temperature (approximately 100°C) and low molar-absorptivity values in the low UV region. These features allow for Brij 35 to be used at the high temperatures which may result from Joule heating and at the low wavelengths which may be required to effect the detection of many compounds.

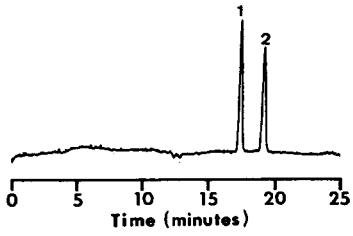

Fig. 2. MEKC separation of benzaldehyde and benzene. Conditions: 80 cm × 100 μm I.D. fused-silica capillary (effective length 50 cm); 0.025 *M* SDS–0.025 *M* Brij 35 in 0.01 *M* Na_2HPO_4 (pH 7.00); +15 kV applied voltage; UV absorbance detection at 215 nm. Peaks: 1 = benzaldehyde; 2 = benzene.

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