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Use of several anionic surfactants for the separation of aniline derivatives in micellar electrokinetic chromatography

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Abstract

The separation of 13 aniline derivatives by micellar electrokinetic chromatography (MEKC) with four anionic surfactants was demonstrated. We used sodium dodecyl sulfate (SDS), sodium *N*-lauroyl-*N*-methyl- β -alaninate (ALE), sodium *N*-lauroyl-*N*-methyltaurate (LMT) and sodium laurylsulfoacetate (LSA) as surfactants. The first application of LSA to MEKC was described. The surfactants having amido linkages, ALE and LMT, gave better separation than SDS. LSA has an ester linkage and its ionic group is sulfonate. Separation with LSA was also better than that with SDS. LSA showed different selectivity from the other surfactants. The effect of the linkages on migration behavior was investigated. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Buffer composition; Surfactants; Anilines

1. Introduction

The migration behavior of solutes is influenced by the nature of surfactant employed in micellar electrokinetic chromatography (MEKC) [1–4]. We have investigated the effect of the surfactant structure on migration behavior in MEKC. In previous work [5], we reported the study of the migration behavior with three homologous sodium *N*-acyl sarcosinates. For hydrophilic solutes, the three sarcosinates gave almost the same separation selectivity.

The structure of the polar moiety of the surfactant is significant in changing the separation selectivity for hydrophilic solutes. We investigated the migration behavior with three kinds of anionic surfactants which have different polar groups [6]. We used

sodium *N*-lauroyl-*N*-methyl- β -alaninate (ALE), sodium *N*-lauroyl-*N*-methyltaurate (LMT) and sodium dodecyl sulfate (SDS). The presence of amido linkages had more effect on their migration behavior than the difference of ionic group. The results were explained by the different contributions of electrostatic interaction among these surfactants.

A new surfactant, sodium laurylsulfoacetate (LSA), was used for the further investigation of the effect of surfactant structures. LSA has an ester linkage and satisfies two requirements: lower Krafft point than ambient temperature and low critical micelle concentration (CMC) value not to degrade efficiency, for using pseudo-stationary phase in MEKC [7]. In this paper, we showed the suitability of LSA as pseudo-stationary phase in MEKC. The separation of aniline derivatives including structural isomers with SDS, ALE, LMT and LSA was investi-

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gated. The effect of the linkages on migration behavior in MEKC was also investigated.

2. Experimental

2.1. Apparatus

MEKC was performed with a CAPI-3000 automated CE system (Otsuka Electronics, Osaka, Japan). A fused-silica capillary (GL Sciences, Tokyo, Japan) of 62 cm (50 cm to the detector) × 50 μm I.D. was used. The instrument control and data collections were performed with a PC-9801 personal computer (NEC Corporation, Tokyo, Japan). Measurement of CMC was performed by conductometry with a CM-5S conductivity meter (TOA Electronics, Tokyo, Japan).

2.2. Reagents

Aniline derivatives, SDS and Sudan IV were obtained from Nacalai Tesque (Kyoto, Japan). AO-10-Dodecylbromide were obtained from Dojin

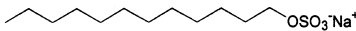
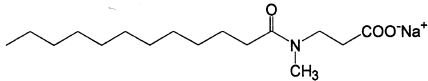
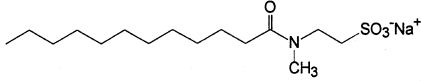
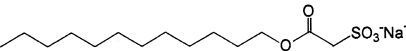
(Kumamoto, Japan). All reagents were of analytical grade and were used without further purification. ALE was donated by Kawaken (Tokyo, Japan) and LMT and LSA were donated by Nikko (Tokyo, Japan). Their purity was checked by elemental analysis. Structures and physicochemical properties of these surfactants in distilled water [7–10] are shown in Table 1. The determined CMC values in the buffer are also shown in Table 1.

2.3. Procedure

Running solutions were prepared by dissolving surfactants with their concentrations at 50 mM in a mixture of 0.02 M sodium dihydrogenphosphate solution and 0.02 M sodium tetraborate solution adjusted to pH 7.0. These solutions were filtered through a 0.45-μm membrane filter prior to use.

Stock solutions of aniline derivatives were prepared in 20% (v/v) methanol in water. Sample solutions for injection were made by ten-fold dilution of the stock solutions with running buffer. Each concentration of solute was about 100 mg/l.

Table 1
Structures and physicochemical properties of surfactants

Surfactant	Krafft point / °C (in distilled water [7–10])	CMC / mM	CMC / mM (in buffer, 30 °C)
Sodium dodecyl sulfate (SDS) 	16	8.6 (40 °C)	4.6
Sodium N-lauroyl-N-methyl-β-alaninate (ALE) 	<20	9.8 (40 °C)	5.0
Sodium N-lauroyl-N-methyltaurate (LMT) 	<0	8.7 (35 °C)	4.5
Sodium laurylsulfoacetate (LSA) 	17	3.8 (40 °C)	1.4

When the running solution was changed, the capillary was rinsed with 0.1 M NaOH for 5 min using a vacuum at the detector reservoir, followed by subsequent rinses of distilled water for 3 min and running buffer for 4 min. After each run, the capillary was rinsed with running buffer for 4 min.

Sample injections were made hydrodynamically, 20 mm×30 s. The injection volume was about 1.7 nl. The set-up voltage and temperature were 20 kV and 30°C, respectively, throughout all experiments. Migrated samples were detected by on-column measurement of UV absorption with photodiode array detection in the range 200–400 nm.

3. Results and discussion

3.1. Separation of aniline derivatives with four surfactants

We selected aniline and mono-substituted isomeric aniline derivatives, anisidines, toluidines, nitroanilines and chloroanilines, as test samples. Their structures and pK_a values [11] are shown in Table 2. They are electrically neutral at pH 7.0.

The chromatogram with SDS is shown in Fig. 1. Some peaks overlapped. ALE and LMT, surfactants having amido linkages, showed different selectivity from SDS for hydrophilic solutes in our previous

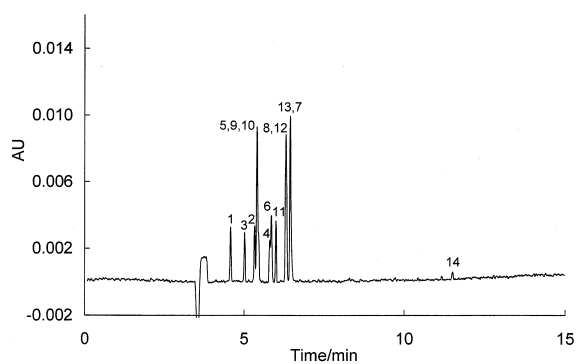


Fig. 1. Chromatogram of aniline derivatives with SDS. Conditions: Capillary, 62 cm (50 cm to the detector)×50 μ m I.D.; concentration of surfactants, 50 mM; running buffer, 0.02 M borate–phosphate (pH 7.0); applied voltage, 20 kV; detection wavelength, 240 nm; temperature, 30°C. Peak identification: 14 = AO-10-dodecylbromide; for other peaks see Table 2.

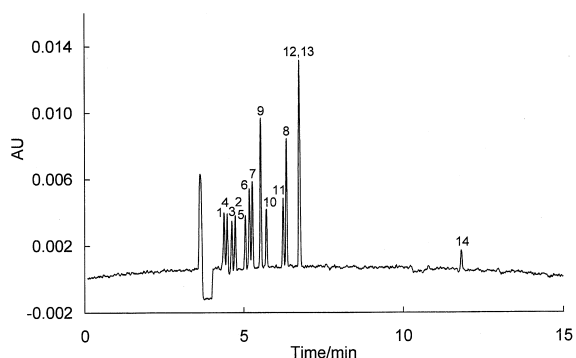


Fig. 2. Chromatogram of aniline derivatives with ALE. Other conditions as in Fig. 1.

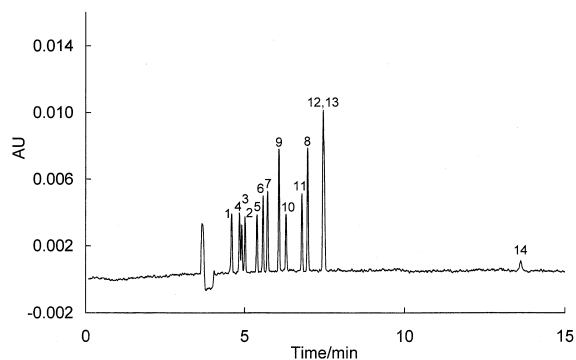


Fig. 3. Chromatogram of aniline derivatives with LMT. Other conditions as in Fig. 1.

Table 2
Structures and pK_a values of investigated aniline derivatives

Compound	Substituent group	pK_a
(1) Aniline	None	4.63
(2) <i>o</i> -Anisidine	<i>o</i> -OMe	4.52
(3) <i>m</i> -Anisidine	<i>m</i> -OMe	4.23
(4) <i>p</i> -Anisidine	<i>p</i> -OMe	5.34
(5) <i>o</i> -Toluidine	<i>o</i> -Me	4.44
(6) <i>m</i> -Toluidine	<i>m</i> -Me	4.73
(7) <i>p</i> -Toluidine	<i>p</i> -Me	5.08
(8) <i>o</i> -Nitroaniline	<i>o</i> -NO ₂	No data
(9) <i>m</i> -Nitroaniline	<i>m</i> -NO ₂	2.466
(10) <i>p</i> -Nitroaniline	<i>p</i> -NO ₂	1
(11) <i>o</i> -Chloroaniline	<i>o</i> -Cl	2.65
(12) <i>m</i> -Chloroaniline	<i>m</i> -Cl	3.46
(13) <i>p</i> -Chloroaniline	<i>p</i> -Cl	4.15

^a Logarithm of the reciprocal of the conjugate acid dissociation constant taken from the literature [11].

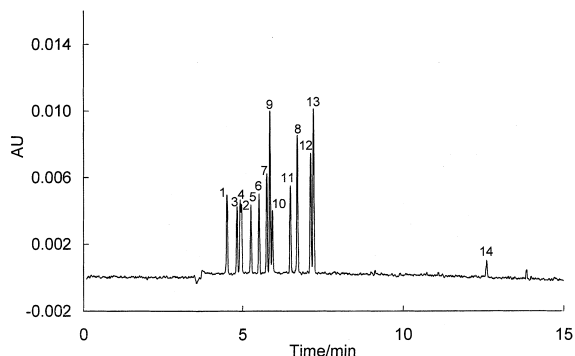


Fig. 4. Chromatogram of aniline derivatives with LSA. Other conditions as in Fig. 1.

study [6]. Therefore ALE and LMT were applied to separate the aniline derivatives. The chromatograms are shown in Figs. 2 and 3. In those cases, all the derivatives except *m*-chloroaniline and *p*-chloroaniline were separated.

In order to separate all aniline derivatives in a single run, we tried to use another surfactant with a different selectivity from ALE, LMT and SDS. We found a new surfactant, LSA, could be used as a pseudo-stationary phase in MEKC. LSA has an ester linkage and a sulfonate ionic group. LSA has a different linkage compared to ALE and LMT. Therefore, LSA is expected to have different selectivity from the other surfactants.

LSA was dissolved in the buffer at ambient temperature and was used as a pseudo-stationary phase in MEKC. The chromatogram is shown in Fig. 4. The separation was almost successful. Separation of *m*-chloroaniline and *p*-chloroaniline was achieved and *o*-anisidine and *p*-anisidine were slightly separated. The selectivity of LSA was different from those of the other surfactants.

3.2. Dependence of the migration behavior on surfactants

In MEKC [1], the capacity factor of an electrically neutral solute is given by:

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

where t_R , t_0 and t_{mc} are the migration time for the solute, electroosmotic flow (EOF) and micelle. Methanol and Sudan IV have been used as a tracer

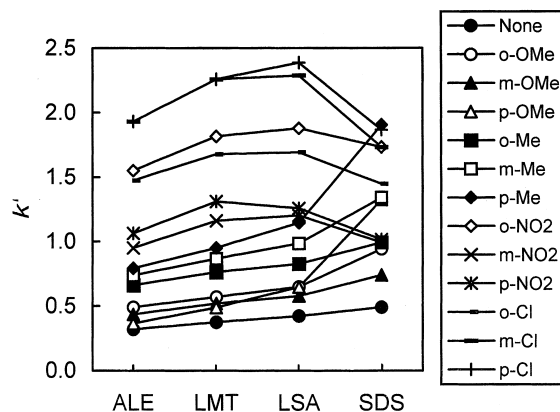


Fig. 5. Dependence of the capacity factors of aniline derivatives on surfactants.

for the EOF and micelle, respectively. In this paper, we used AO-10-dodecylbromide as a tracer for the micelle [12]. It gave a single peak and the migration time agreed with that of the peak of Sudan IV. Three sets of measurements were made for all runs and the average values were used in the paper. Relative standard deviations (R.S.D.s) of the migration time and the capacity factor of solutes were within 1.8% and 2.1%, respectively.

Figs. 5 and 6 show the dependence of the capacity factors and the separation factors of aniline derivatives on the surfactants. The capacity factors with ALE were slightly smaller than those with LMT, but the separation selectivity with ALE was almost the same as that with LMT. The capacity factors and separation factors with SDS were in

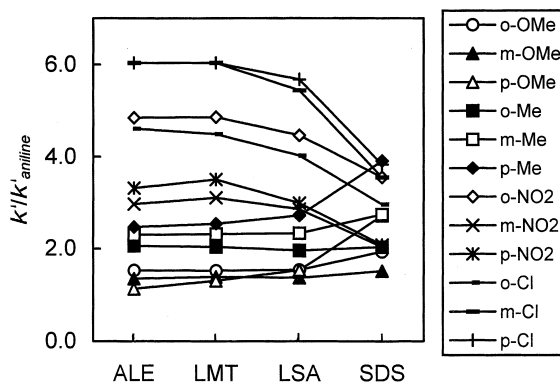


Fig. 6. Dependence of the separation factors of aniline derivatives on surfactants.

contrast to those with the other surfactants. LSA has a moderate dependence between LMT and SDS.

The migration behavior was affected by the existence and the type of linkages. *p*-Anisidine, *m*-toluidine and *p*-toluidine have relatively higher pK_a values than those of the other aniline derivatives and they most strongly interacted with the SDS micelle. Therefore, their migration behavior seemed to be affected by electrostatic interaction.

In order to confirm the effect of electrostatic interaction, the dependence of the selectivity difference between surfactants on the pK_a of solutes was investigated. We calculated the values of the logarithms of the ratio of capacity factors between ALE and the other surfactants. The result of these calculations is shown in Fig. 7.

In the normalization with ALE, SDS was most sensitive to the pK_a of solutes. A slight dependence was observed for LMT. LSA showed a moderate behavior between LMT and SDS. These data indicate that the existence of the linkages in the structure of surfactant suppresses the electrostatic interaction between the surfactants and solutes. The effect of ester linkage is smaller than that of amido linkage.

Another tendency of the selectivity was found from these plots. For chloroanilines and nitroanilines, their capacity factors with LMT and LSA were greater than those with ALE. On the contrary, those with SDS were almost the same as those with ALE. This result indicates that the selectivity difference between surfactants is based on not only the effect of electrostatic interaction but also on other effects.

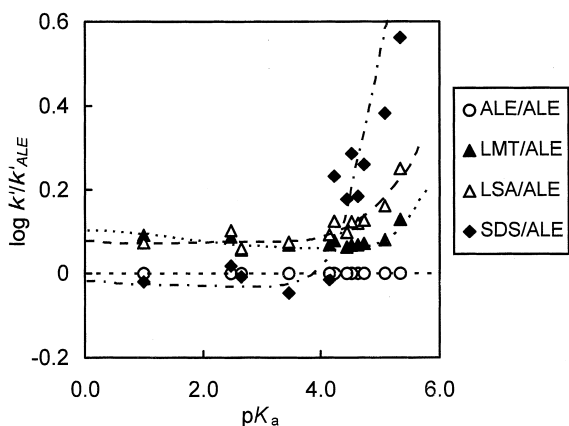


Fig. 7. Dependence of the selectivity difference between surfactants on the pK_a of solutes.

The concept of LSER (linear solvation energy relationship) is applied to investigate the migration behavior in MEKC [3]. This is a useful method for the general recognition, however, specific situation such as isomeric separation cannot be explained by the concept only. For example, all solvatochromic parameters of toluidine isomers are the same [13], but they can be separated in MEKC. Further investigation is necessary to discuss the separation mechanism in MEKC in detail.

4. Conclusions

LSA was suitable for use as pseudo-stationary phase in MEKC. The migration behavior with LSA was compared to those with ALE, LMT and SDS. In the separation of investigated aniline derivatives, LSA showed different selectivity from the other surfactants and gave the best separation. The use of these surfactants may enable one to control the separation selectivity of hydrophilic solutes in MEKC.

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