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Enhancements in micellar electrokinetic chromatographic separations with decanonyl-N-methylalkanamide micelles through the addition of alkyl sulfates

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Abstract

Sodium dodecyl sulfate (SDS) and sodium tetradecyl sulfate (STS) are effective additives for improving the efficiency of the micellar electrokinetic chromatography (MEKC) with micelle forming solutions of decanonyl-N-methylglucamide (MEGA 10) with boric acid, pH 10. MEGA 10, a nonionic surfactant, is one of a series of micelle forming compounds for which the surface charge density of the micelle and electrophoretic migration behavior can be varied through the extent of complexation with a charged species, i.e., borate or boronate ions. Using aniline and a series of 11 substituted anilines as model solutes, the addition of 0–10 m*M* concentrations of SDS or 0–8 m*M* STS to buffers containing 20 m*M* or 40 m*M* MEGA 10 and boric acid (1:4 concentration ratio) results in an increase in the solute capacity factors and an improvement in separation efficiencies with only small changes in the rate of electroosmotic flow and size of the elution range. Further addition of the sodium alkyl sulfate surfactants results in a large expansion of the elution range. The effects of SDS and STS are attributed to changes in the structure of the MEGA 10/borate micelles resulting in a mixed surfactant micelle structure which is more permeable for the solutes.

Keywords: Micelles; Buffer composition; Decanonylmethylalkanamides; Anilines

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a powerful method for the separation of neutral solutes and for enhancing the selectivity of ionic solutes [1]. It is one member of the family of methods which are collectively referred to as capillary electrophoresis (CE) and which have rapidly become accepted for a wide range of analytical applications. As in all CE methods, MEKC separations occur in small bore capillaries over which a large electric field is applied [2]. In the case of MEKC, ionic micelles present in the running buffer migrate at a different velocity from that of the bulk electrolyte solution. Separations are affected by differences in the partitioning of solutes between the pseudostationary phase composed of the micelles and the surrounding aqueous phase. Also like all CE methods, MEKC offers the advantages of rapid, efficient, separations with small sample volumes and small solvent consumption for a wide variety of solutes. The recent demonstration of CE separations

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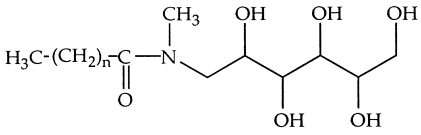
on small, highly miniaturized instruments microfabricated on a single glass chip only further suggests CE methods should have a significant impact for clinical and environmental field analysis for many years [3].

In MEKC the migration times of neutral solutes fall within an elution range ($t_{mc}-t_0$). The boundaries of the elution range are formed by the migration time of species unretained by the micelle and migrating with the electroosmotic flow (EOF), t_0, and the migration time of the micelles for species fully solubilized by the micelles, t_{mc}. The magnitude of the elution window is one of the most important factors for determining the separation efficiency and the overall analysis time. Unfortunately, for the commonly used sodium alkyl sulfate and alkyltrimethylammonium halide surfactants the magnitude of the elution range is largely unaffected by the ionic strength of the buffer or variations in the buffer pH within the useful range appropriate for these systems. The elution range can be expanded for these commonly used surfactant through the addition of simple organic solvents [4] such as methanol and 1-propanol, etc. or by silanizing the interior surface of the fused-silica capillary [5] which in both cases reduces the rate of bulk flow in the capillary, the EOF.

In order to retain the magnitude of the elution range as an adjustable parameter useful for the optimization of MEKC separations, El Rassi and co-workers [6–12] have extensively demonstrated the utility of in situ charged micelles. The surface charges of these micelles are based on the extent of the complexation between neutral surfactants bearing polyolic polar head groups and charged species such as borate or boronate ions. Consequently the surface charge density of the micelles is variable by changing the concentration of borate or boronate, the concentration of surfactant or the pH of the running buffer. Increasing the surface charge density of the micelles results in an increase in the rate of migration of the micelles, v_{mc}, in the direction opposite to the EOF, a decrease in the net migration rate of the micelles ($v_{net}=v_{EOF}+v_{mc}$) and an expansion of the elution range. To date, four such neutral surfactant types, alkyl-glucosides [6,8,11], alkylglucoamides [7,8,12], alkylmaltosides [8,9] and alkanoylsucroses [8,9], have been investigated for their utility in MEKC.

Of these surfactant types, the alkylglucoamide (MEGA) surfactants are the most readily available due to their application in the solubiliztion of cellular membrane components [13]. As depicted below, MEGA 8 ($n=6$), MEGA 9 ($n=7$) and MEGA 10 ($n=8$) contain a linear sugar head group connected to an alkyl tail through an amide linkage. The free rotation of the carbon atoms forming the linear sugar head group leads to a good bonding geometry for the facile complexation with the tetrahydroxyborate ions formed from boric acid in alkaline solutions [7]. While the aggregation numbers for micelles formed from the MEGA surfactants are unavailable, of the three surfactants listed above, MEGA 10, with the longest alkyl chain in the series, has the lowest critical micelle concentration, 6–7 m*M* [14].



decanoyl-N-methylglucamide

In this work we have examined the effects of the addition of sodium *n*-alkyl sulfates (SxSs) on MEKC separations with MEGA 10/borate micelles leading to the formation of mixed surfactant micelles. We have previously shown that hydrophobic counterions inducing disorder in the structure of sodium dodecyl sulfate (SDS) micelles can have beneficial effects for MEKC separations [15]. The formation of mixed surfactant micelles is another simple way to induce structural disorder in the micelles which can alter the retention behavior of the pseudostationary micellar phase and improve a separation. Experiments utilizing mixed surfactant micellar systems have been reported for both the capillary zone electrophoresis of proteins [16] and the MEKC of small molecular solutes [17–22]. For the latter case, both anionic–zwitterionic mixed micelles and anionic–nonionic mixed micelles have been shown to be capable of either improving separation efficiencies [17,21] or increasing the elution range dramatically [22].

We have examined the effects of the addition of both SDS and sodium tetradecyl sulfate (STS) added to MEGA 10/borate micelles at levels less than the MEGA 10 concentration. The test solutes employed are aniline and a series of simple substituted anilines.

We focus on this class of solutes because they provide solutes covering a range of polarities and are an important class of compounds both as industrial chemicals and as environmental hazards. At additive concentration levels below 12 m*M* the net effect of the SxS surfactants is to alter the retention behavior of the MEGA 10/borate micelles while having only a small effect on magnitude of the elution widow which remains largely a function of the MEGA 10/boric acid ratio.

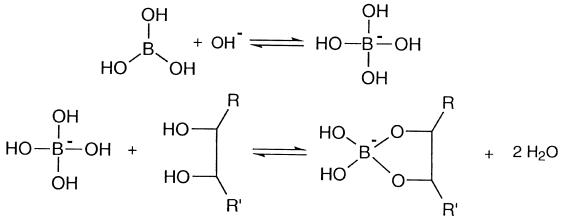
2. Experimental

The experiments reported herein were performed using a Beckman Pace Model 5500 CE instrument. Separations were carried out in a fused-silica capillary, 67 cm×50 μm I.D.×375 μm O.D. with detection at 60 cm (Polymicro Technologies, Phoenix, AZ, USA) maintained at 25°C. Prior to each separation the capillary is rinsed with 0.1 *M* NaOH (10 min), water (4 min) and the running buffer (5 min). The separation voltage was +15 kV and the eluting solutes are detected by UV absorption at 254 nm due to absorbance of MEGA 10 at shorter wavelengths. The running buffers employed consist of 20 m*M* or 40 m*M* decanonyl-N-methylglucamide (MEGA 10) (Sigma, St. Louis, MO, USA), 80 m*M* or 160 m*M* boric acid (Aldrich, Milwaukee, WI, USA) and varying amounts of SDS or STS (both from Lancaster Synthesis, Windham, NH, USA). The pH of the running buffers is adjusted to 10.0±0.1 using a 1 *M* NaOH solution.

All anilines used were reagent grade and obtained from Aldrich, Kodak (Eastman Kodak, Rochester, NY, USA), Lancaster Synthesis or the Sigma and used as received. The 12 anilines were dissolved in Milli-Q water (Millipore, Bedford, MA, USA) at a level of 100 ppm each. Samples were prepared by diluting the aniline mixture in an equal volume of the 20 m*M* MEGA 10/80 m*M* boric acid running buffer and also containing Sudan III (Sigma) which has a weak absorbance at 254 nm and serves as the marker for the migration time of the micelles. Samples were injected using a high pressure injection for 8 s. All data reported reflect at least two replicate separations recorded for each different composition of the running buffer.

3. Results and discussion

The surface charge density of MEGA 10 micelles in pH 10 boric acid media is a result of the complexation of tetrahydroxy borate ions with the polyol portion of the surfactant head group. Consequently the two equilibria depicted below are important in the in situ adjustment of the micelle surface charge density which effects the electrophoretic migration behavior of the MEGA 10/borate micelles and determines the elution range. The first equilibria is the pH dependent formation of the tetrahydroxy borate ion and the second equilibria is the borate–polyol complexation for which the energetically favored reaction with the *cis*-1,2-diol is depicted [23]. In this investigation experiments are primarily conducted with pH 10 buffers with the ratio of MEGA 10 to boric acid held constant at 1:4 to assess the effect of the alkylsulfate surfactants on the elution range and the partitioning of the anilines into the mixed surfactant micelles.



The micellar electrokinetic chromatograms of a 12 aniline mixture from three separations conducted in pH 10 buffers containing only MEGA 10 with boric acid are pictured in Fig. 1. In Fig. 1A the buffer composition is 20 m*M* MEGA 10 and 80 m*M* boric acid and the resulting separation gave baseline resolution for four of the 12 anilines. An expanded view of this chromatogram, which is chosen as a reference to which comparisons can be drawn, appears in Fig. 2A. In Fig. 1B the concentrations of both MEGA 10 and boric acid are doubled to 40 m*M* and 160 m*M*, respectively, resulting in a expanded elution range but still only the baseline separation of four of the 12 anilines. An expanded view of this chromatogram appears in Fig. 5A. For the chromatogram pictured in Fig. 1C the buffer composition is 20 m*M* MEGA 10 with 160 m*M* boric acid. As expected, halving the ratio of MEGA 10 to boric acid increases the surface charge density of the

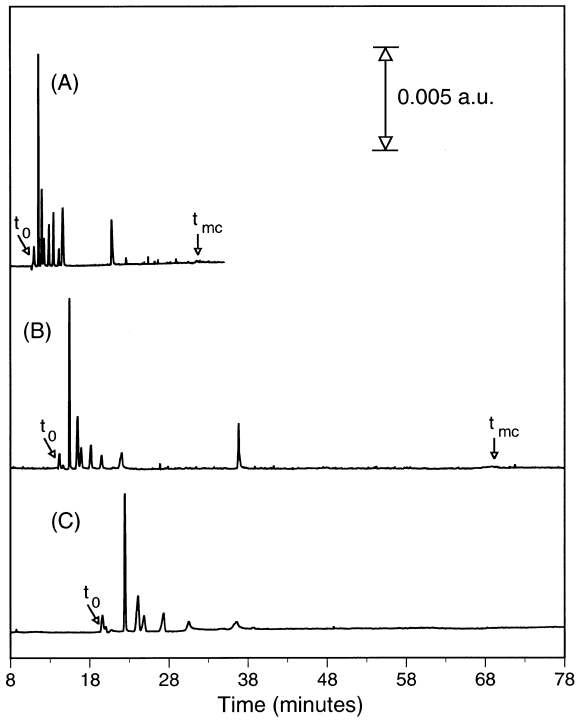


Fig. 1. Micellar electrokinetic chromatograms of a mixture of 12 anilines obtained in pH 10 buffers consisting of (A) 20 m*M* MEGA 10 and 80 m*M* boric acid, (B) 40 m*M* MEGA 10 and 160 m*M* boric acid and (C) 20 m*M* MEGA 10 and 160 m*M* boric acid. Sample solution ~50 ppm of each aniline + Sudan III; capillary, 67 cm (60 cm to detector) × 50 μm I.D. × 375 μm O.D.; +15 kV applied voltage; detection at 254 nm.

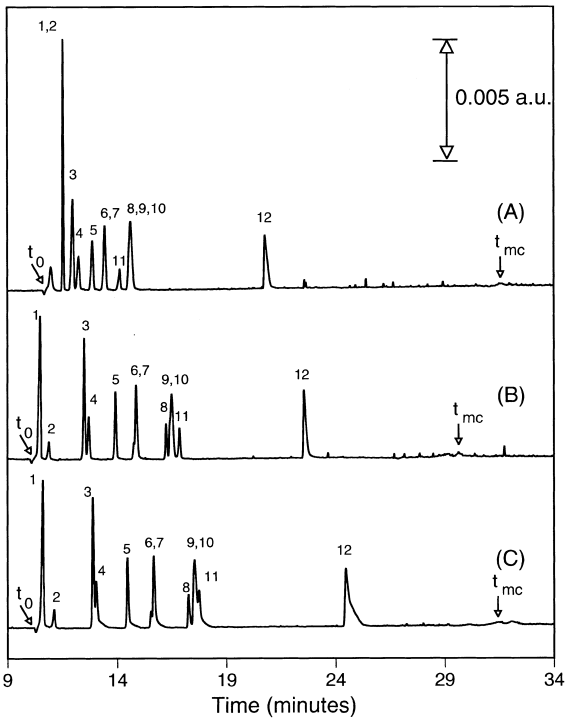


Fig. 2. Micellar electrokinetic chromatograms of a mixture of 12 anilines obtained in pH 10 buffers consisting of (A) 20 m*M* MEGA 10 and 80 m*M* boric acid, (B) 20 m*M* MEGA 10, 80 m*M* boric acid and 10 m*M* SDS and (C) 20 m*M* MEGA 10, 80 m*M* boric acid and 8 m*M* STS. Sample solution, ~50 ppm of each aniline + Sudan III; capillary, 67 cm (60 cm to detector) × 50 μm I.D. × 375 μm O.D.; +15 kV applied voltage; detection at 254 nm. Solutes are identified according to the numbers given in Table 1.

micelles and results in a large increase in the elution range (>100 min) and also in this case operating currents in excess of 140 μA (15 kV separation voltage), extensive band broadening and a loss of separation efficiency.

Pictured in Fig. 2 are two micellar electrokinetic chromatograms of the 12 aniline mixture with either 10 m*M* SDS or 8 m*M* STS added to the pH 10, 20 m*M* MEGA 10/80 m*M* boric acid running buffer along with a chromatogram in the absence of alkyl sulfate. The identities of the 12 anilines are listed in Table 1 along with their pK_b values, octanol–water partition coefficients which are an indicator of the relative hydrophobicity of the compounds, and an identification number for the chromatograms in this paper. The chromatograms in Fig. 2B,C are taken from a series of experiments in which the concentration of SDS and STS buffer was increased from 0–16 m*M* and 0–12 m*M*, respectively. Relative to the experiments without the addition of SDS or STS, Fig. 1A, the addition of 10 m*M* SDS or 8 m*M* STS results in an improvement in the separation with the baseline resolution of eight of the 12 anilines for SDS and seven of the 12 for STS. Some improvement in the separation is realized upon the addition of lesser concentrations of SDS and STS with only small changes in the size of the elution range.

The variation of the size of the elution range as a function of the concentration of SxS additives is apparent in Fig. 3 where the average values of t_0 and t_{mc} for the complete series of experiments with 20 m*M* MEGA 10/80 m*M* boric acid buffers with SDS and STS are plotted. For the experiments with SDS the addition of 0–10 m*M* concentrations results in a

Table 1
Anilines investigated with pK_b, octanol–water log P values and compound numbers

Aniline	pK_b[a]	Octanol–water log P[b]	Compound No.
Aniline	9.37	0.9	**2**
2-Bromoaniline	11.47	2.11	**7**
3-Bromoaniline	10.42	2.10	**8**
4-Bromoaniline	10.14	2.26	**9**
4-Chloroaniline	9.85	1.88	**6**
3,4-Dichloroaniline	11.25	2.69	**12**
4-Isopropylaniline	n.a.	2.40	**11**
2-Nitroaniline	14.26	1.85	**5**
3-Nitroaniline	11.53	1.37	**3**
4-Nitroaniline	13	1.39	**4**
Sulfanilamide (pK_a=10.43)	11.64	−0.26	**1**
3-Trifluoromethylaniline	11.40	2.29	**10**

[a] The values listed are from Refs. [25–27].
[b] Values taken from Refs. [28,29].

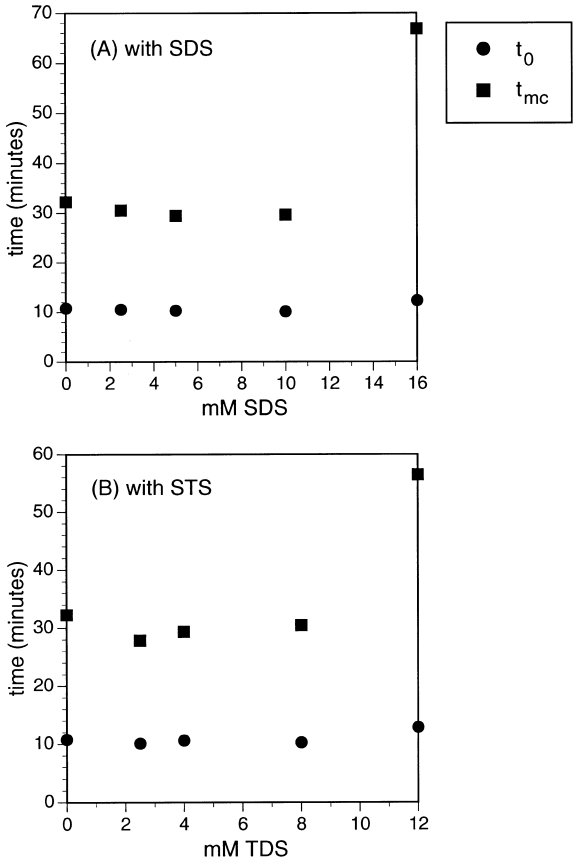


Fig. 3. Plots of the average values of t_0 and t_{mc} as a function of the concentration of (A) SDS and (B) STS added to buffers containing 20 mM MEGA 10 and 80 mM boric acid.

decrease in the elution range of less than 11% and in the case of STS the addition of up to 8 mM results in a maximum decrease in the elution range of less than 13%. The addition of higher concentrations of the alkyl sulfates, 16 mM SDS and 12 mM STS results in a large expansion of the elution range to ≈55 min and ≈43 min, respectively. As a point of comparison, the elution range in pH 10, 100 mM boric acid/NaOH buffer containing 30 mM SDS in the same capillary with a +15 kV separation voltage is ≈45 min.

Also evident in the chromatograms in Fig. 2 is the increased retention capability of the mixed surfactant micelles due to the addition of the SxS surfactants. To better illustrate the effects of the formation of mixed surfactant micelles on the solute retention in Fig. 4 we have plotted the quantity $-RT \ln k'$ as a function of SxS additive concentration for the 11 neutral anilines in the test mixture. Sulfanilamide (**1**), which is negatively charged in pH 10 solutions is excluded. For these plots the capacity factors, k', are calculated according to Eq. (1)

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

where t_0 is identified in each run by a recognizable disturbance observed in the baseline signifying the EOF and t_{mc} is obtained from the migration time of Sudan III. The largest relative standard deviation (R.S.D.) in the determination of any k' is 5% but

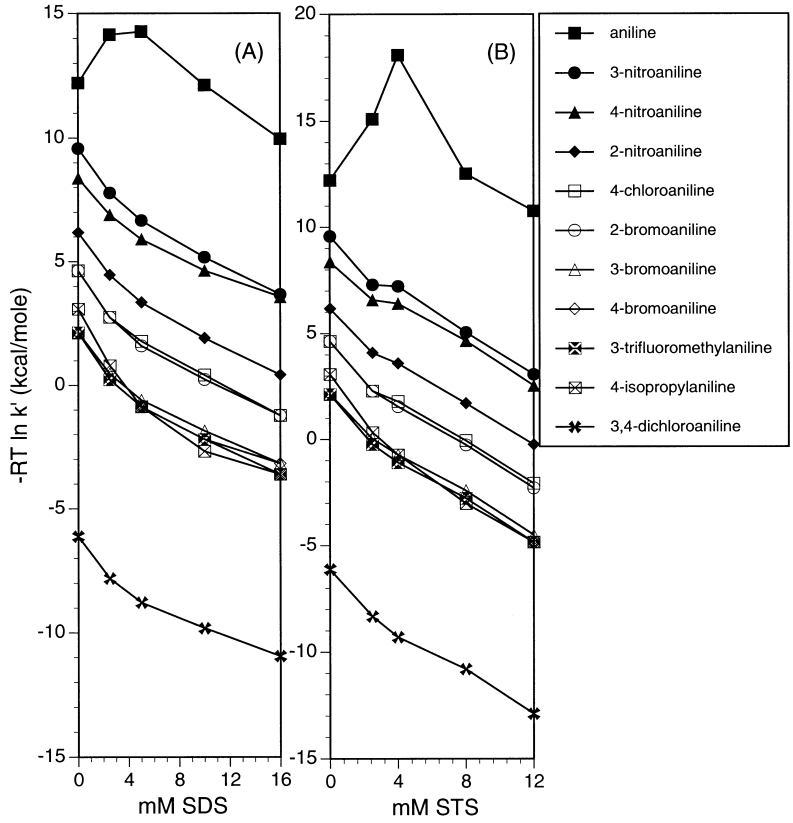


Fig. 4. Plots of the quantity $-RT$ ln k' for a series of separations with increasing concentrations of (A) SDS and (B) STS added to buffers containing 20 m*M* MEGA 10 with 80 m*M* boric acid. Only data for the 11 neutral anilines is included, data for sulfanilamide is excluded.

more typical uncertainties are less than 1%. The difference with and without SxS added, $-RT\,\Delta\ln k'$, represents both the change in transfer free energy of solute to the micelles and changes in the phase ratio (V_s/V_m) due to the incorporation of SxS ions in the micelles. As shown in Fig. 4, both SxS surfactants have a similar effect on the retention behavior of the solutes. The addition of either SDS or STS leads to a consistent increase in the retention all neutral anilines with the exception of aniline (**2**). Overall the retention of aniline by the micelles is poor, lessened by the addition of low concentrations of SxS ions and marginally increased at the highest SxS additive concentrations investigated. In addition Fig. 4 reveals a change in the elution order for 4-isopropylaniline (**11**) in the presence of SxS ions resulting in an elution order for the 11 neutral anilines in better agreement with that predicted from the octanol–water log *P* values listed in Table 1.

The beneficial effects of the addition of SDS and STS are also in evidence in experiments with buffers containing a higher concentration of MEGA 10. Pictured in Fig. 5 are three micellar electrokinetic chromatograms of the 12 aniline mixture obtained in a 40 m*M* MEGA 10/160 m*M* boric acid running buffer and in the same buffer with either 10 m*M* SDS (Fig. 5B) or 8 m*M* STS (Fig. 5C). As in the experiments with 20 m*M* MEGA 10/80 m*M* boric acid, the addition of the SxS ions and the formation of mixed surfactant micelles results in an improvement in the solute retention without a large change in the elution range. In particular, whereas the experiments without SxS ions (Fig. 5A) result in the baseline separation of five of the 12 anilines the

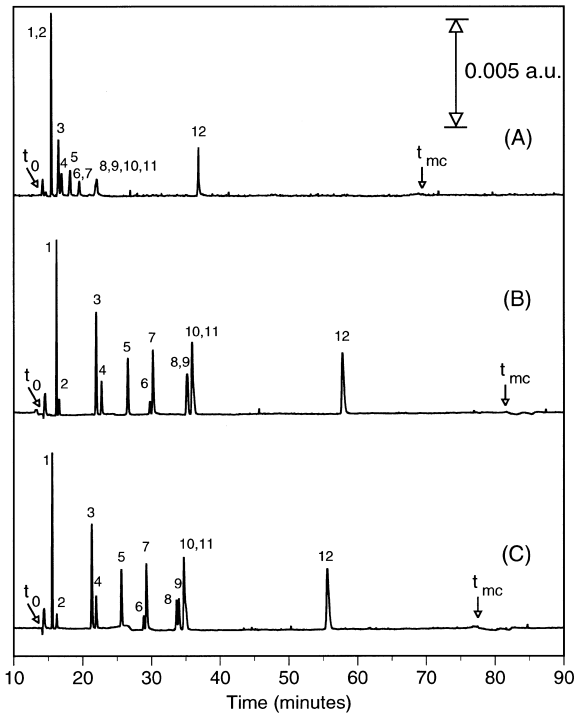


Fig. 5. Micellar electrokinetic chromatograms of a mixture of 12 anilines obtained in pH 10 buffers consisting of (A) 40 m*M* MEGA 10 and 160 m*M* boric acid, (B) 40 m*M* MEGA 10, 160 m*M* boric acid and 10 m*M* SDS and (C) 40 m*M* MEGA 10, 160 m*M* boric acid and 8 m*M* STS. Sample solution, ~50 ppm of each aniline + Sudan III; capillary, 67 cm (60 cm to detector) × 50 μm I.D. × 375 μm O.D.; +15 kV applied voltage; detection at 254 nm. Solutes are identified according to the numbers given in Table 1.

addition of 10 m*M* SDS and 8 m*M* STS results in the near-baseline separation of eight and 10 anilines, respectively with at most a 15% increase in the elution range.

Separation efficiencies are also much improved upon the addition of the SxS surfactants. Listed in Table 2 are the number of theoretical plates per meter based on the width at half-height of the solute peaks for three representative anilines from the mixture calculated for each of the buffers associated with the chromatograms in Figs. 2 and 5. The three solutes, 3-nitroaniline (**3**), 2-nitroaniline (**5**) and 3,4-dichloroaniline (**12**) were chosen to represent solutes migrating at early, late and in the middle of the elution range and because they are well resolved in all separations. In experiments with either 20 m*M* MEGA 10/80 m*M* boric acid and 40 m*M* MEGA 10/160 m*M* boric acid an improvement in separation efficiency by a factor of 2 to 3 is realized upon the addition of 8 m*M* TDS or 10 m*M* SDS to the buffer. A comparison of the data for the two separations without the addition of the SxS surfactants reveals that the improvement in efficiency is not merely a function of a higher surfactant concentration but is a result of the inclusion of SxS surfactants in the MEGA 10 based buffers.

In the case of both SDS and STS we attribute the improvements in separation efficiency to the formation of mixed surfactant micelles. As evidenced by the size of the elution range, the mixed surfactant

Table 2
Separation efficiencies for three representative anilines in the test mixture

MEKC buffer composition	Separation efficiencies (plates/m)		
	3-Nitroaniline (**3**)	2-Nitroaniline (**5**)	3,4-Dichloroaniline (**12**)
20 m*M* MEGA 10/80 m*M* BA[a]	$8.1 \cdot 10^4$	$9.2 \cdot 10^4$	$1.3 \cdot 10^5$
with 8 m*M* STS	$1.4 \cdot 10^5$	$2.3 \cdot 10^5$	$2.3 \cdot 10^5$
with 10 m*M* SDS	$1.8 \cdot 10^5$	$1.7 \cdot 10^5$	$1.8 \cdot 10^5$
40 m*M* MEGA 10/160 m*M* BA	$6.3 \cdot 10^4$	$5.7 \cdot 10^4$	$5.6 \cdot 10^4$
with 8 m*M* TDS	$1.5 \cdot 10^5$	$1.9 \cdot 10^5$	$1.8 \cdot 10^5$
with 10 m*M* SDS	$1.7 \cdot 10^5$	$1.9 \cdot 10^5$	$2.0 \cdot 10^5$

[a] BA = Boric acid.

micelles retain much of their size and surface charge density character at low to modest additive levels but are substantially different at higher SxS concentration levels. It is expected that the mixed surfactant micelle structure is not a reflection of an ideal mixture because previous studies of mixed nonionic–anionic surfactant micelles show strong evidence for the segregation of the surfactant types within the micelles [24]. Based on the anticipated segregation of the surfactants and the difference in size of the nonpolar portions of SDS and STS relative to that of MEGA 10 we expect a considerable amount of structural disorder is present in the mixed surfactant micelles which results in a deeper penetration of the aniline solutes into the micelles as well as less hindered transfer of the solutes in and out of the micelles leading to the overall improvement in the separation.

Our results for mixed MEGA 10/SxS micelles are similar to those reported in MEKC studies with mixed anionic–zwitterionic surfactant micelles [21]. In this work mixed surfactant micelles of N-dodecyl-N,N-dimethylammonium-3-propane-1-sulfonic acid (SB-12) with SDS in which the SB-12 concentration levels were kept below the concentration of SDS were observed to increase the capacity factors for a range of analytes, improve the separation efficiencies by a factor of 2 to 4, with only a minimal reduction in the elution range and rate of EOF. Separations in which the concentration of SB-12 was in excess of the SDS concentration resulted in a reduction in the elution range and solute capacities.

In summary we have shown using a mixture of aniline and 11 simple substituted anilines that an improvement in the efficiency of MEKC separations with in situ charged micelles of MEGA 10 can be realized from the addition of the alkyl sulfate surfactants SDS and STS. The improvement results from changes in the character and structure of the mixed surfactant micelles relative to those containing only MEGA 10. As demonstrated in experiments with either 20 m*M* MEGA 10/80 m*M* boric acid or 40 m*M* MEGA 10/160 m*M* boric acid electrolytes, the addition of SxS ions at concentrations less than 12 m*M* results in an increase in the capacity factors for all neutral solutes with the exception of aniline while only small changes are observed in the rate of EOF and the size of the elution range. Additionally a two- to three-fold increase in the separation efficiencies for all solutes investigated is realized upon the addition of SxS ions to the MEGA 10 based buffers.

Acknowledgements

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