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Effects of tetraalkylammonium salts on the micellar electrokinetic chromatography of aniline and substituted anilines

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Abstract

Experiments investigating the effects of tetraalkylammonium (TAA) ions on the micellar electrokinetic chromatography (MEKC) of aniline and a series of 12 substituted anilines using sodium *n*-dodecyl sulfate (SDS) are reported. Increasing the concentration of tetramethylammonium (TMA) ions results in increases in the elution range ($t_{mc}-t_0$) and the migration times for the aniline solutes. In contrast the addition of small concentrations of tetrabutylammonium (TBA) ions leads to changes in the elution order and a considerable improvement in the separation of the anilines without a significant increase in the elution range. Further increases in the concentration of TBA ions to levels above 1/3 the concentration of SDS narrows the elution range and has a detrimental effect on the separation. The effects of the TBA ions are attributed to changes in the structure of the SDS micelles resulting in a more open and disordered SDS micelle which enhances the transfer of anilines with polar ring substituents into the micelle. © 1998 Elsevier Science B.V

Keywords: Buffer composition; Anilines; Tetraalkylammonium ions

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 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 this work we have used MEKC for the separation of a series of anilines. Aniline and substituted anilines are an important class of compounds both as

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industrial chemicals and as environmental hazards. These compounds are the feedstocks for a variety of dyes, resins, varnishes and urethane polymers. Aniline itself is used as a vulcanization accelerator in the rubber industry. In the area of agrochemicals, aniline and its derivatives are very important in the production of a variety of widely used carbamate, dinitroaniline and phenylurea herbicides. Following the application of these types of herbicides to crops, degradation via dealkylation or hydrolysis results in the introduction of anilines into the environment. Since many anilines have solubilities in water in excess of 100 ppm and are known to be blood poisons and toxins capable of attacking the liver and nervous system, new and simple methods for their separation and detection are of interest.

Two reports applying CE methods to the separation of anilines have recently appeared in the literature. Okafo et al. [4] have employed free zone capillary electrophoresis (CZE) to the separation of anilinium ions using low pH buffers and exploited the increased basicity of the anilines in deuterium oxide (^2H_2O) based buffers. Brumley and Jones [5] compared the separation of 16 neutral anilines using a MEKC method with a pH 8.5 borate buffer containing 100 m*M* sodium cholate micelles and 10% acetone to the separations of the same anilines with capillary gas chromatography (GC). The addition of acetone in the later experiment as an organic modifier improved the selectivity in the separation. Other additives used in MEKC separation include cyclodextrins which affect the separations of highly hydrophobic compounds [6] and other simple organic solvents such as methanol and 1-propanol, etc., to reduce the rate of bulk flow in the capillary (EOF) and extend the elution range. Additionally, the elution range can also be extended by silanating the interior surface of the fused-silica capillary [7].

In this and the previously reported applications of CE to the analysis of anilines the detection was accomplished using UV absorption (at 214 nm), the most common method of detection used in CE. Aromatic amines such as anilines are relatively easily oxidized and amperometric methods are also applicable to this class of analytes [8,9]. Amperometric detection can be an attractive detection alternative to UV absorption because of its superior sensitivity with detection limits approaching the single attomole range for easily oxidized species [10]. Preliminary experiments in our laboratory suggest detection limits of 1 femtomole are easily achievable for many anilines [11]. Because the oxidation potentials of anilines are lower in high pH electrolytes and because high concentrations of surfactant can have a deleterious effect on amperometric detection [10] we have chosen to explore the addition of tetraalkylammonium ions to enhance the selectivity of the MEKC separation of anilines at high pH and with relatively low surfactant concentrations.

The use of TAA ions as ion pairing reagents is common in both CE and HPLC for the separation of ionic species. In CE they have been used to enhance the separation efficiency for a variety of ionic analytes such as water soluble vitamins, penicillin and cephalospolin antibiotics in MEKC separations with SDS [12], and in the CZE of organic acids and bases and the products resulting from glycoprotein digests [13]. The ability of TAA ions to associate with neutral species has also been used to affect the separation of polyaromatic hydrocarbons in CZE experiments employing predominantly organic electrolytes [14]. Our work with TMA and TBA ions shows these additives to also be quite useful for enhancing the separation of neutral anilines in aqueous buffers containing sodium *n*-dodecyl sulfate (SDS) albeit through different mechanisms.

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., detection at 60 cm, Polymicro Technologies, Phoenix, AZ, USA) maintained at 25°C. Prior to each separation the capillary was rinsed with 0.1 *M* NaOH (3 min) and the running buffer (4 min). The separation voltage was +15 kV and the eluting solutes were detected by UV absorption at 214 nm. The running buffers employed consisted of a 33 m*M*, pH 10.0±0.1, boric acid–NaOH solution containing 12 m*M*, 24 m*M* or 48 m*M* SDS (Aldrich, Milwaukee, WI, USA) and varying amounts of tetrabutylammonium bromide (TBAB) or tetramethylammonium bromide (TMAB) (both from Eastman Kodak, Rochester, NY, USA).

All anilines used were reagent grade and obtained from Aldrich, Kodak, Lancaster Synthesis (Windham, NH, USA), or Sigma (St. Louis, MO, USA) and used as received. The 13 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 33 m*M* boric acid–NaOH solution with 12 m*M* SDS and also containing Sudan III (Sigma) which serves as the marker for the migration time of the micelles, t_{mc}. 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

3.1. Separations with buffers containing SDS only

Pictured in Fig. 1 are three representative micellar electrokinetic chromatograms for the 13 anilines used in this study obtained in pH 10 borate–NaOH buffers containing 12, 24 or 48 m*M* SDS only. The identities of the 13 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 peaks in the chromatograms in this paper. While the separation is improved with increasing the SDS concentration, the complete separation of the 13 anilines is not achieved with 48 m*M* SDS, the highest concentration used in this study. Three pairs of solutes coeluted 4-methylaniline (**3**) with 3-nitroaniline (**4**), 4-chloroaniline (**6**) with 2-nitroaniline (**7**) and 4-bromoaniline (**10**) with 3-trifluoromethylaniline (**11**). As expected the elution range expanded with increasing the concentration of SDS from 12 m*M* to 48 m*M* [$(t_{mc}-t_0)$ increases from 13.0 to 18.1 min] but the selectivity did not improve significantly. A change in the order of migration for aniline (**1**) and sulfanilamide (**2**) was observed as the SDS concentration increased from 12 m*M* to 48 m*M*. Sulfanilamide has a pK_a of 10.43 and is negatively charged at pH 10. Consequently it does not interact strongly with the negatively charged micelles and migrates at a rate relatively unaffected by the presence of SDS.

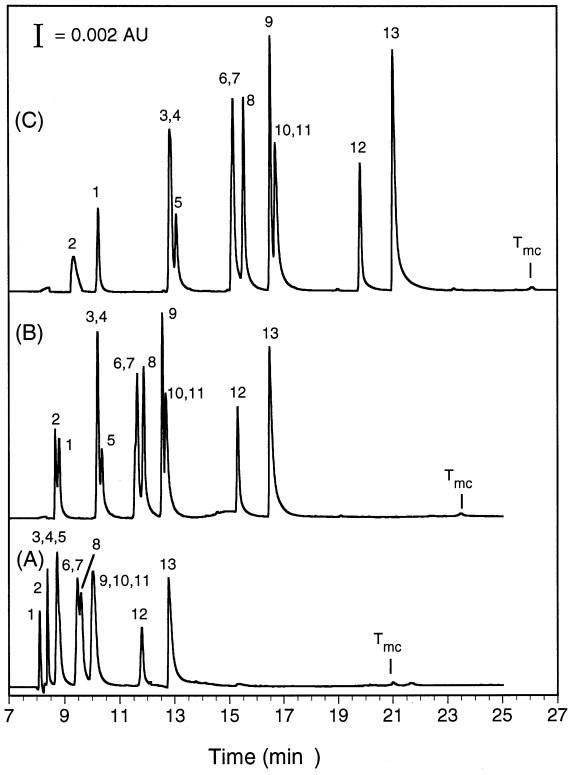

Fig. 1. MEKC chromatograms of 13 anilines in buffers containing (A) 12 m*M*, (B) 24 m*M* and (c) 48 m*M* sodium *n*-dodecyl sulfate. Sample solution: ~50 ppm of each aniline + Sudan III; capillary: 67 cm × 50 μm; buffer: 33 m*M* borate–NaOH (pH 10); voltage: +15 kV; detection: UV absorption at 214 nm. Solutes are identified according to the numbers given in Table 1.

3.2. Separations with buffers containing SDS and tetramethylammonium ions

To investigate the effects of TMA ions on the separation of the 13 anilines in Table 1 a series of experiments were conducted with increasing concentrations of TMAB added to running buffers containing either 12, 24 or 48 m*M* SDS. As shown in Fig. 2A a considerable improvement in the separation relative to that with SDS only is realized upon the addition of the first 4 m*M* of TMAB with near baseline resolution of 11 of the 13 anilines. The improvement in the separation quality which continues with additional increases in the amount of TMAB added is mainly the result of the expansion of the elution range. Plotted in Fig. 3A is the ratio (t_{mc}/t_0) as a function of TMAB concentration added.

Table 1
Anilines investigated with pK_b, octanol–water log P values and chromatogram identification number

Aniline	pK_b[a]	Octanol–water log P[b]	Peak No.
Aniline	9.37	0.9	1
2-Bromoaniline	11.47	2.11	8
3-Bromoaniline	10.42	2.10	9
4-Bromoaniline	10.14	2.26	11
4-Chloroaniline	9.85	1.88	6
3,4-Dichloroaniline	11.25	2.69	13
4-Isopropylaniline	n.a.	2.40	12
4-Methylaniline	8.9	1.38	3
2-Nitroaniline	14.26	1.85	7
3-Nitroaniline	11.53	1.37	4
4-Nitroaniline	13	1.39	5
Sulfanilamide (pK_a=10.43)	11.64	−0.26	2
3-Trifluoromethylaniline	11.40	2.29	10

[a] The values listed are from Refs. [20–22].
[b] Values taken from Refs. [23,24].

For each of the three different SDS concentrations investigated, increasing the amount of TMAB added to the running buffer results in an expansion of the elution range and at approximately the same rate.

A considerable fraction of the increase in the elution range can be attributed to the decrease in the rate of EOF resulting from the addition of TMA ions. Considering the experiments with 48 mM SDS as an example. Without the addition of TMAB the υ_{EOF} is 6.9 cm/min and $\upsilon_{Sudan\ III}$, the net migration rate of the micelles, is 2.2 cm/min. This gives a migration rate of the micelles, υ_{mc}, equal to −4.7 cm/min where the minus sign indicates that the migration is opposite to the direction of EOF. Following the addition of 16 mM TMAB the rates are υ_{EOF}=6.3 cm/min, $\upsilon_{Sudan\ III}$=1.4 cm/min and υ_{mc}=−4.9 cm/min. This data reveals that over the 0–16 mM TMAB concentration range investigated, where the elution range increases by 40%, only a 4% change in migration rate of the micelles is observed while the rate of EOF decreases by 9%.

The modest effect of TMA ions on improving the selectivity of the separation is consistent with increasing the elution range by modifying the EOF. Pictured in Fig. 4 are the capacity factors (k') determined for the 12 neutral anilines investigated in experiments using running buffers containing 48 mM SDS. The capacity factors are calculated according to

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

where t_0 is identified in each run by a recognizable disturbance observed in the baseline signifying the EOF [15] and t_{mc} is obtained from the migration time of Sudan III. The largest relative standard deviation in the determination of any k' is 5% but more typical uncertainties are less than 1%. As shown in Fig. 4, over the 0–16 mM TMAB concentration range investigated there is no change in the order of migration of the neutral anilines and generally only a gradual increase in the migration times is observed which leads to the improved separations. Plots of the capacity factors for experiments with 12 and 24 mM SDS are similar to the experiments with 48 mM SDS with the principle difference being the overall reduction in k' values as expected for lower surfactant concentrations.

For the 0–16 mM TMAB concentration range investigated with either 12, 24 or 48 mM SDS, the best separations are achieved with the maximum amount of TMAB in the running buffers. A typical MEKC chromatogram under these conditions is pictured in Fig. 2B. The quality of the separation is much improved compared to that obtained when the running buffer contains only SDS (Fig. 1C) but the separation is still incomplete with 4-chloroaniline

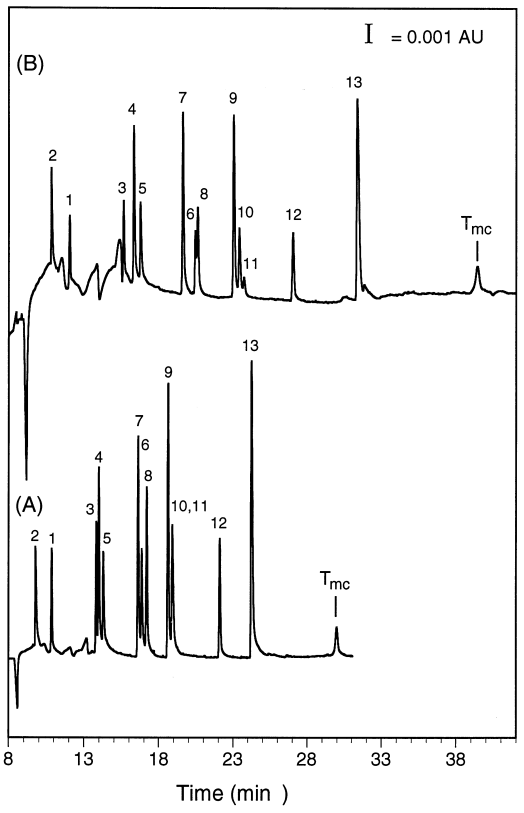

Fig. 2. MEKC chromatograms of the 13 anilines in buffers containing 48 m*M* sodium *n*-dodecyl sulfate and (A) 4 and (B) 16 m*M* tetramethylammonium bromide. Sample solution: ~50 ppm of each aniline+Sudan III; capillary: 67 cm×50 μm; buffer: 33 m*M* borate–NaOH (pH 10); voltage: +15 kV; detection: UV absorption at 214 nm. Solutes are identified according to the numbers given in Table 1.

continuing to comigrate with 2-bromoaniline. In addition, the overall separation time is greater than 30 min.

3.3. Separations with buffers containing SDS and tetrabutylammonium ions

In contrast to TMAB, the addition of concentrations of TBAB<4 m*M* significantly improves the quality of the separation of the 13 anilines. Pictured in Fig. 5 is a series of MEKC chromatograms with 24 m*M* SDS and 1–8 m*M* TBAB. With 2 or 4 m*M* TBAB added to the 24 m*M* SDS running buffer all 13 anilines are at least partially separated and nine anilines are baseline resolved. However further increases in the TBAB concentration to 8 m*M* and higher in the experiments with 24 m*M* SDS results in a decrease in the quality of the separation as reflected by the merging of aniline migration times. A similar decrease in the quality of the separation was observed in running buffers containing 12 m*M* SDS and TBAB concentrations above 4 m*M*.

The effect of TBAB on the separations with 48 m*M* SDS is much the same. As shown in Fig. 6, experiments with 48 m*M* SDS and 4 m*M* result in the baseline separation of 11 anilines and the partial separation of the remaining two. This separation is superior to that obtained in buffers containing 48 m*M* SDS and 16 m*M* TMAB (Fig. 2B) and in a time only ~2 min longer than the separation with SDS alone (Fig. 1C).

The effect of increasing the concentration of TBAB on the width of the elution range is more complicated than in the case of TMAB. Plotted in Fig. 3B is the ratio (t_{mc}/t_0) as a function of TBAB concentration for the experiments with 12, 24 and 48 m*M* SDS. Following a decrease in (t_{mc}/t_0) with the initial addition of 1 m*M* TBAB, further increases in the TBAB concentration result in an expansion of the elution range followed in the cases of 12 and 24 m*M* SDS by a contraction in the elution range when the TBAB concentration is greater than 1/3 the concentration of SDS. As evidenced by the experiments with 24 m*M* SDS, the migration rates without TBAB are $\upsilon_{EOF}=7.0$ cm/min, $\upsilon_{Sudan\ III}=2.5$ cm/min and $\upsilon_{mc}=-4.5$ cm/min and with 16 m*M* TBAB added are $\upsilon_{EOF}=7.0$ cm/min, $\upsilon_{Sudan\ III}=2.8$ cm/min and $\upsilon_{mc}=-4.5$ cm/min indicating the contraction can be attributed to a decrease in the migration rate of the micelles. The decrease in the separation quality at large TBAB concentrations coincides with the contraction in the elution range.

That TBAB is affecting the separation in a manner different than TMAB is revealed in the plots of the calculated capacity factors as a function of TBAB concentration. These plots for experiments with 24 and 48 m*M* SDS are depicted in Fig. 7A,B. These results are qualitatively in agreement with the results from experiments with 12 m*M* SDS. As shown in Fig. 7, a change in the migration order is observed for 4-chloroaniline (**6**) with 2-bromoaniline (**8**) and there is steady movement of 4-isopropylaniline (**12**)

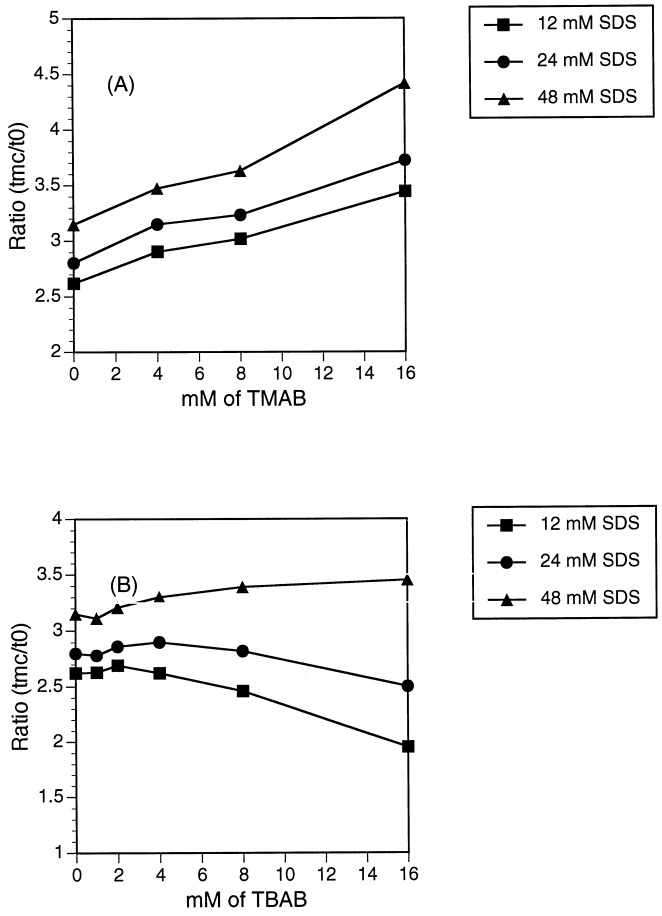

Fig. 3. Plots of the elution window (t_{mc}/t_0) as a function of the concentration of (A) tetramethylammonium bromide and (B) tetrabutylammonium bromide.

up in the migration order. Evidence for both these observations can be clearly seen in the chromatograms depicted in Fig. 5. Two other trends observed in the capacity factor versus TBAB concentration plots which are different than those observed in the experiments with TMAB are a decrease in the migration time for 4-methylaniline (**3**) in the experiments with 48 m*M* SDS and the rapid and large increase in the capacity factors for 3,4-dichloroaniline (**13**) which is observed at all three levels of SDS concentration.

Previous studies of the effect of TAA salts on the MEKC separations of cationic, anionic and zwitterionic species using SDS micelles at the 50 m*M* level also revealed differences between TMA ions and TBA ions [12]. For anionic analytes migration times were observed to increase with the addition of 20 m*M* TMAB but were affected much less by the addition of an equal concentration of TBAB. In the case of cationic and zwitterionic species, the addition of 20 m*M* TMAB had a small effect on the migration times whereas the same amount of TBAB resulted in much less retention by the micelles and much shorter migration times. As indicated in Ref. [12], the effects of the TAA salts on the migration of charged solutes in MEKC separations are the result of the balance between complexation of the solute with the TAA ion and the complexation of the TAA ion with the micelle, the later resulting in changes in the size and character of the micelles. In the present work sulfanilamide (**2**) is the only ionic solute and its migration is relatively unaffected by the presence of

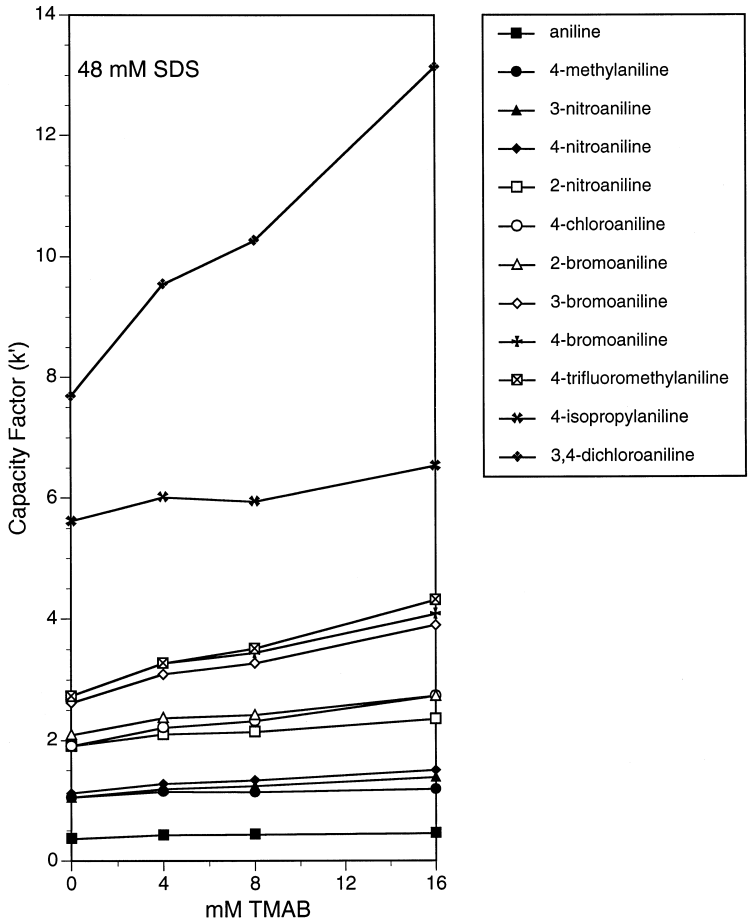

Fig. 4. Capacity factors for a series of separations with increasing concentrations of tetramethylammonium bromide in buffers containing 48 m*M* sodium *n*-dodecyl sulfate. Only data for the 12 neutral anilines are included, data for sulfanilamide are excluded.

TBA ions suggesting that complexation is not a factor in these experiments.

In SDS micelle solutions with only sodium counterions present, the negatively charged sulfate head groups are uniformly positioned on the surface of the micelle and the alkyl chains forming the hydrophobic core are in predominantly extended conformations. Substitution of TMA ions for the sodium ions creates a more disordered structure of the head groups on the micelle surface [16–19]. The head groups are spaced further apart and are displaced vertically with respect to one another increasing the micelle surface roughness and the thickness of the head group layer. The alkyl chains in the core of the micelle are also more loosely packed with the TMA ions leading to significant chain bending and water penetration [17]. Relative to the TMA ion, the effects of the longer chain TBA ion are expected to be greater as a result of both enhanced binding of the more hydrophobic counterion to the micelle and the larger C_4 spacer group inserted into the micelle [16,19].

Our results suggest that the gradual replacement of some of the sodium counterions with the hydrophobic TBA counterions opens voids in the micelle structure allowing for greater penetration of solutes into the micelles. To better illustrate the effects of TBA ions we have plotted the quantity $-RT\ \ln(k')$ as a function of TAA additive concentration in Fig. 8. The difference with and without TAA additive, $-RT\ \Delta\ln\ (k')$, represents the change in transfer free

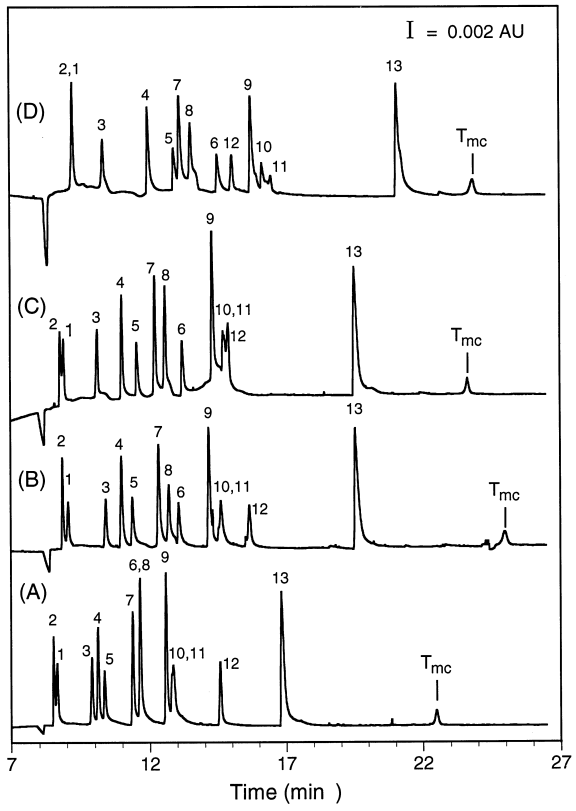

Fig. 5. MEKC chromatograms of the 13 anilines in buffers containing 24 m*M* sodium *n*-dodecyl sulfate and (A) 1, (B) 2, (C) 4 and (D) 8 m*M* tetrabutylammonium bromide. Sample solution: ~50 ppm of each aniline+Sudan III; capillary: 67 cm×50 μm; buffer: 33 m*M* borate–NaOH (pH 10); voltage: +15 kV; detection: UV absorption at 214 nm. Solutes are identified according to the numbers given in Table 1.

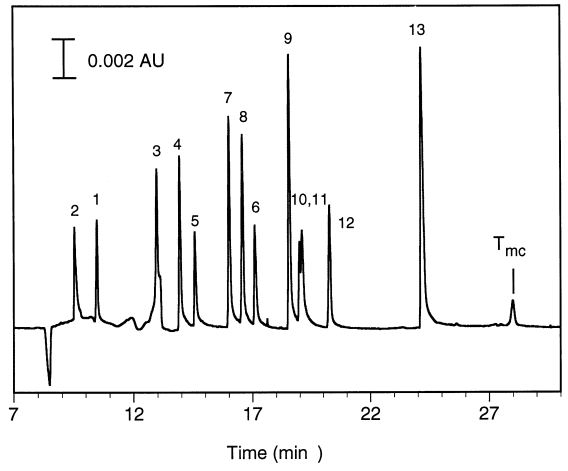

Fig. 6. MEKC chromatogram of the 13 anilines in buffers containing 48 m*M* sodium *n*-dodecyl sulfate and 4 m*M* tetrabutylammonium bromide. Sample solution: ~50 ppm of each aniline+Sudan III; capillary: 67 cm×50 μm; buffer: 33 m*M* borate–NaOH (pH 10); voltage: +15 kV; detection: UV absorption at 214 nm. Solutes are identified according to the numbers given in Table 1.

energy of solute to the micelles due to the presence of the TAA counterions. As shown in Fig. 8A, the addition of TBAB leads to a substantial reduction in the transfer free energy for all neutral anilines with the exception of 4-methylaniline (**3**) and 4-isopropylaniline (**12**). The reduction is also larger for those anilines with polar substituents in the 3- and/or 4-position which suggests a preferred orientation of the solute for penetration into the micelle. In contrast, a similar plot for the addition TMAB shown in Fig. 8B reveals much smaller reductions in transfer free energy which are more uniform for all 12 neutral anilines.

The two solutes which are clearly affected differently by the addition of TBAB are 4-methylaniline (**3**) and 4-isopropylaniline (**12**), two anilines with nonpolar substituents. These anilines are also the most basic anilines in this study as the pK_b of 4-methylaniline is 8.9 and the pK_b for 4-isopropylaniline is expected to be nearly the same. Data for both solutes reveal an increase in the transfer free energy upon the addition of increasing concentrations of TBAB. This would be expected if the retention behavior for these solutes is largely the result of a shallow penetration of the solute into the micelle and interactions between the alkyl substituents in the 4-position on the aniline with those methylene groups in the micelle near the sulfate head groups. These interactions would be most perturbed through the structural disorder induced by the TBA counterions and subsequent changes in polarity inside the micelle due to increased water penetration.

4. Conclusion

TAA ions are effective additives for improving the separation of anilines in MEKC separations with

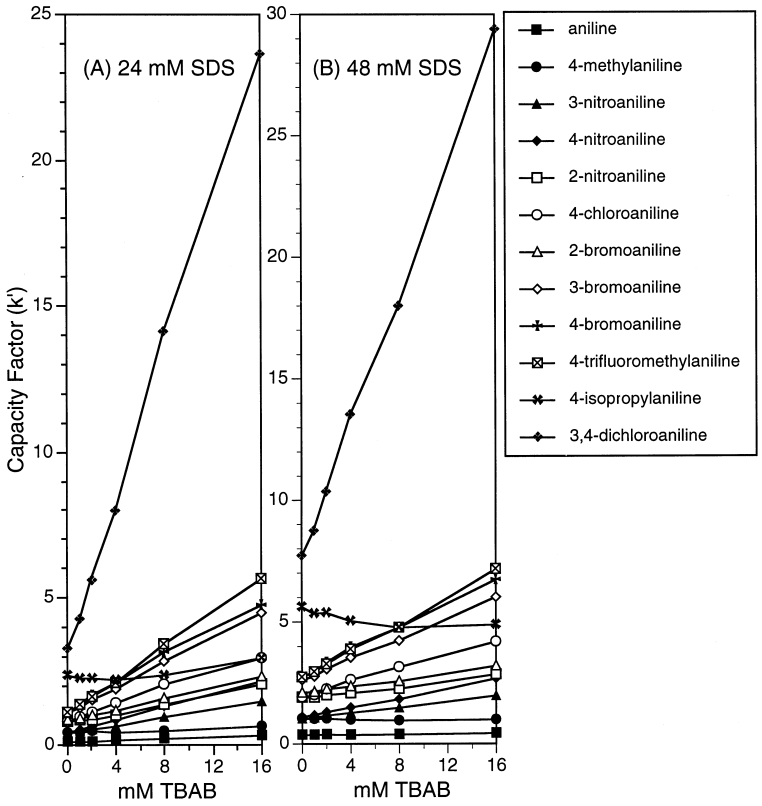

Fig. 7. Capacity factors for a series of separations with increasing concentrations of tetrabutylammonium bromide added to buffers containing (A) 24 and (B) 48 m*M* sodium *n*-dodecyl sulfate. Only data for the 12 neutral anilines are included, data for sulfanilamide are excluded.

SDS micelles. The addition of increasing amounts of TMAB results in a decrease in the rate of EOF, an increase in the elution range and a gradual increase in the solute migration times. More discriminating changes in the selectivity of the separation with SDS micelles are achieved through the addition of TBAB to the running buffer. The addition of TBAB at levels below 1/3 of the SDS concentration results in an improvement in the quality of the separation without a large increase in the elution range. The effects of TBAB are the result of the creation of defects in the structure of the SDS micelles due to the replacement of the some of the sodium counterions with TBA ions. These changes in the micelle structure accelerate the transfer of anilines with polar substituents into the micelles while having a small decelerating effect on anilines with nonpolar substituents. Continued increases in the TBAB concentration above 1/3 the SDS concentration results in a decrease in the elution range and degradation of the quality of the separation due to a decrease in the electrophoretic velocity of the micelles [20–24].

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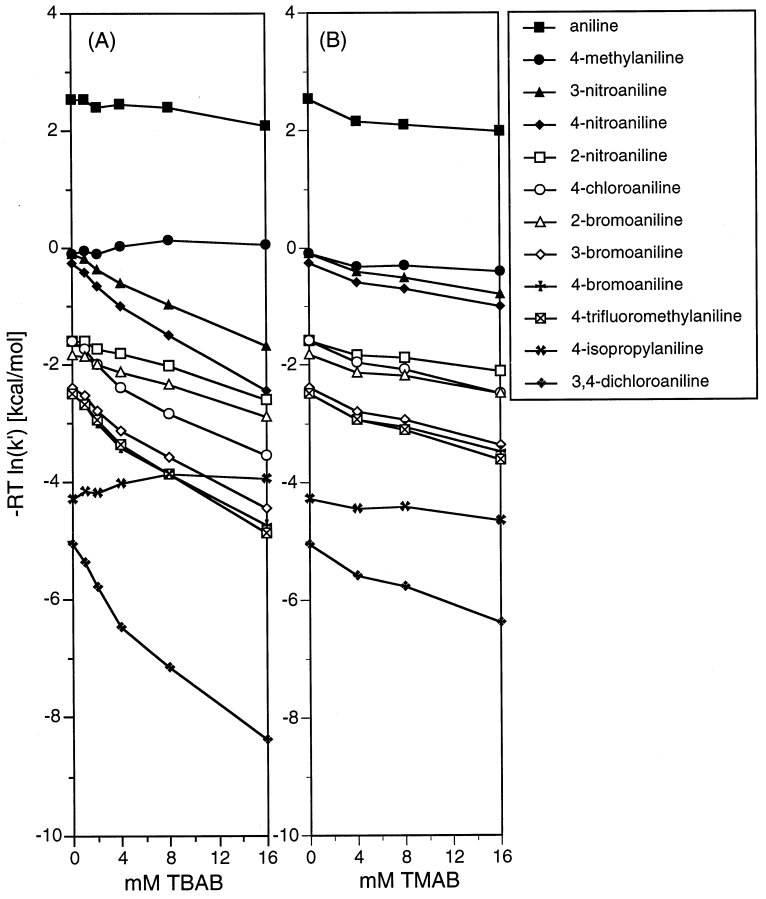

Fig. 8. Plots of the quantity, *RT* ln (*k′*), for a series of separations with increasing concentrations of (A) tetrabutylammonium bromide and (B) tetramethylammonium bromide added to buffers containing 48 m*M* sodium *n*-dodecyl sulfate. Only data for the 12 neutral anilines are included, data for sulfanilamide are excluded.

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