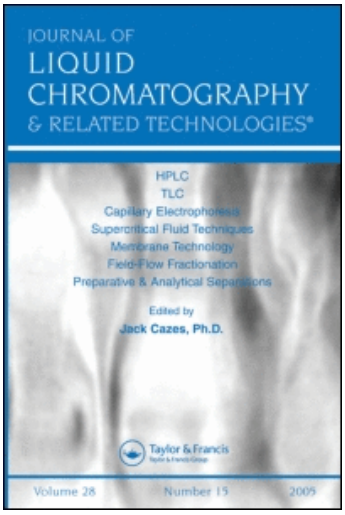

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Micellar Electrokinetic Chromatography Using Mixed Sodium Dodecyl Sulfate and Sodium Cholate

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MICELLAR ELECTROKINETIC CHROMATOGRAPHY USING MIXED SODIUM DODECYL SULFATE AND SODIUM CHOLATE

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ABSTRACT

The migration behavior of fifteen dansylated amino acids, two estrogens, and two polycyclic aromatic hydrocarbons (PAHs) was studied by micellar electrokinetic chromatography (MEKC) with mixed micelles. The buffer system consisted of 10 mM borate at pH 9.0, containing 50 mM total surfactant concentration. Five different ratios of sodium dodecyl sulfate (SDS) and sodium cholate (SC) surfactants were used, namely: 100:0, 75:25, 50:50, 25:75, and 0:100 (SDS:SC). The numbers refer to molar ratio percent. The migration time window was 50% larger in 50:50 (SDS:SC) compared to 100% SC, and 15% larger than that of 100% SDS.

The migration times of PAHs and estrogens exhibited a pattern similar to that of Sudan III in all the SDS/SC mixed micellar systems, due to their hydrophobic property. Although most amino acids are negatively charged at pH 9, their migration patterns were different depending on their R-group.

The migration times of amino acids with negatively charged R-groups increased continuously with increasing percent of SC in the SDS/SC mixed micellar systems, while the migration time of amino acids with positively charged R-group continuously decreased with increasing percent of SC in the SDS/SC mixed micellar system. For other groups of amino acids, the migration times in SDS/SC mixed micellar systems exhibited variation without specific pattern.

In general, the use of SDS and SC mixed micelles extended the migration time window to some extent, but it was not sufficient to get satisfactory resolution for the amino acids tested in this study.

INTRODUCTION

Capillary Electrophoresis (CE) is a highly efficient micro separation technique which can resolve charged compounds under the influence of a gradient electric field.[1] Micellar electrokinetic chromatography (MEKC) is a variation of CE whereby a micelle is added to the running buffer to effect the separation of uncharged compounds. Today, MEKC is used for the separation of both ionic and neutral molecules. The separation mechanism in MEKC is based on the differential partitioning of a molecule between the micellar pseudo-stationary phase and the aqueous phase.[2] There are many surfactants that can be used in MEKC. They can be grouped into four classes based on the charge characteristics of the head group, namely: anionic, cationic, neutral and zweitterionic. Normally, in MEKC one surfactant is added to the buffer to effect the separation. Modifiers, such as organic solvents,[3,4] cyclodextrins,[5] urea,[6,7] are added to the micellar buffer to enhance the separation. The addition of an additive to a micellar solution may: (a) affect the distribution of the solutes between the aqueous and the micellar phases; (b) shift the micellization equilibrium; (c) affect the additive-surfactant interactions in the aqueous and micellar phases; and (d) affect the aggregation number, shape and degree of ionization. This becomes especially prominent when two different micelles are used in the same buffer solution where solubilization of the solute in the micellar system may be affected. For example, it was observed that cholesterol was more soluble in mixed micelle compared to micelles of single bile salt.[8] The use of mixed micelle[9,10] resulted in significant extension of the elution window, and an improvement in resolution. Also, it influenced the retention of the solutes. Rasmussen et al.[11] were able to resolve benzene from benzaldehyde by using mixed Brij 35/SDS micelles. Little and Foley[12] were able to optimize their separation by changing the concentration of the

micelle. Ahuja et al.[10] studied the effect of changing the concentration of Brij 35 while keeping the concentration of SDS constant on the retention and resolution of n-alkylphenone homologues, using coated and uncoated fused silica capillaries. In both types of columns a plot of molar concentration of Brij 35 versus the reciprocal of electroosmotic and electrophoretic velocities resulted in a linear relationship which increased with increasing the molar concentration of Brij 35 while SDS molar concentration remained constant at 0.02 M. Bumgarner and Khaledi[9] used a mixed micellar system made of bile salt surfactants at different mole fractions as well as total micelle concentrations in order to optimize the resolution of a mixture of corticosteroids which were not resolved when SDS alone was used. Optimum separation was achieved using a ternary surfactant system made of 50 mM each of dehydrocholate, taurocholate and SDS. They also reported that the addition of SDS to a mixture of bile salt micelles resulted in a significant extension of the elution window.

The use of bile salt surfactants in combination with SDS is interesting because of the type and properties of the micelles that each group forms. SDS forms a spherical micelle, while bile salts forms helical micelles. The SDS micelle consists of a hydrophobic inner core and a charged external shell, while the bile salt micelle is the reverse, the hydrophobic region faces the outside while the hydrophilic region faces inward. Therefore, a mixture of a bile salt and a SDS will form a micellar system which possesses micelles with both hydrophobic and hydrophilic outer surfaces facing not only the aqueous buffer but the solutes in the buffer solution, which gives a micellar system that can be used to resolve mixtures of hydrophilic and hydrophobic compounds. This is an advantage not available using only a single surfactant.

The objective of this research is to study the effect of a binary mixed micelle system on the migration times of a group compounds having different physical/chemical properties. In this study, the binary mixed micelles SDS and sodium cholate (SC) were selected. Also three different groups of compounds of different chemical and physical properties, namely, two polycyclic aromatic hydrocarbons (fluoranthene and naphthalene), two estrogens (estrone and estriol), and dansylated amino acids were selected. The amino acids were divided into six groups: (a) polar but uncharged R group (serine and glutamine); (b) negatively charged R group (aspartic, glutamic, and cysteic acid); (c)positively charged R group (lysine and arginine); (d) hydrophobic with an aliphatic R group (alanine, isoleucine, and leucine); (e) hydrophobic with an aromatic R group (phenyalanine, tryptophan and tyrosine); and amino acids with special R group (glycine and proline).

EXPERIMENTAL

Chemicals

Dansylated amino acids and sodium cholate were purchased from Sigma Chemical Company (St. Louis, MO). estriol and estrone were purchased from Steraloids, Inc. (Wilton, NH). and fluoranthene and naphthalene were obtained from Aldrich Chemical Company (Milwaukee, WI). Sodium dodecyl sulfate (SDS) was from Fluka (Ronkonkoma, NY). All other chemicals for preparation of the electrolyte buffer were purchased from Fisher Scientific (Fair Lawn, NJ). Deionized water was generated from a Nanopure Water system manufactured by Barnstead/Themolyne (Dubuque, IA). All electrolyte solutions were filtered through a 0.45 μm nylon acrodisc filter purchased from Gelman Science (Ann Arbor, MI) and degassed.

Apparatus and Procedures

A Beckman CZE Model P/ACE 5510 equipped with diode array detector, an automatic injector, a fluid-cooled column cartridge and a System Gold data station were used in this study. All runs were performed at 25° C, using a 57 cm x 50 μm (50 cm to detector) fused silica capillary from Polymicro Technologies, Inc. (Phoenix, AZ). Each new capillary was washed with 0.1 N NaOH and deionized water then equilibrated with the buffer for 20 min. Capillary electrophoresis was operated at 18 kV. The resulting current ranged from 52-75 μA. The buffers with different concentration of sodium cholate (SC) and sodium dodecylsulfate (SDS) were prepared fresh in deionized water. Solutes were dissolved in the buffer system to be used. was specified in the figure legends. Injections were made using the pressure mode for 5 s at 0.5 psi. Dansylated amino acids, naphthalene and fluoranthene were detected at 200 nm while estrone and estriol were detected at 280 nm.

The running buffer throughout the experiment was prepared as borate buffer 10 mM, pH 9.0. SDS and SC were mixed in five ratios, namely: 100:0, 75:25, 50:50, 25:75, and 0:100 (SDS/SC). The numbers refer to molar ratio percent. In all, the total surfactant concentration was 50 mM. with total concentration of 50 mM. Solutes were dissolved in the buffer solution for injection. Sudan III was dissolved in methanol, then mixed with buffer solution containing the desired SDS/SC mixed ratios, which were then injected to determine the migration time of aqueous buffer solution (t_o) and micelles (t_{mc}). The capillary was rinsed with running electrolyte for 3.5 min between runs.

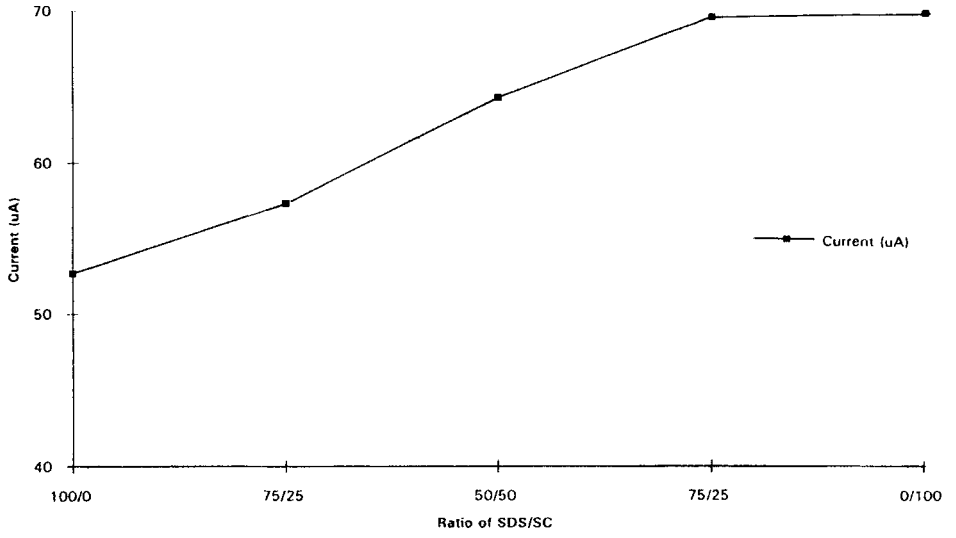

Figure 1. Current versus ratio of SDS/SC in mixed micelles. Instrument: Beckman Model P/ACE System 5510; Column: Bare fused-silica; Column dimensions: L_{total}=57cm; $L_{detector}$=50cm; i.d.=50um; Buffer: 10 mM borate, plus 50 mM surfactant in single or mixed micelles; pH=9.0; applied voltage: 18 kV.

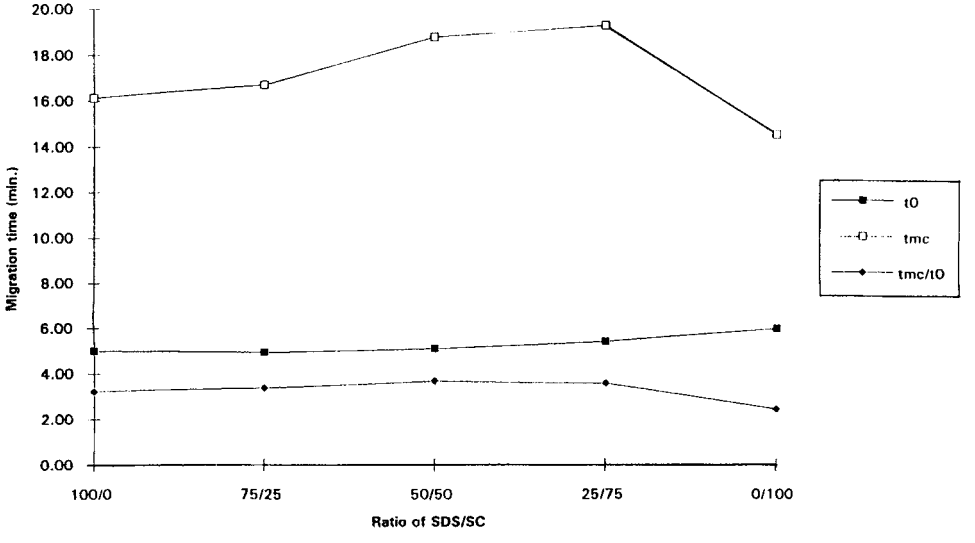

Figure 2. Effect of ratio of SDS/SC on the migration times of methanol (t_o) and Sudan III (t_{mc}) in MEKC. Experimental conditions as in Figure 1.

When different surfactant ratio systems were employed, the capillary was rinsed in sequence with water, 0.1 M NaOH, water, and running electrolyte 2 min. each. Samples were run in triplicates. Data of migration time were the average of three replications.

RESULTS AND DISCUSSION

Effect of Mixed Micelles on Current

The effect of mixing SDS and SC surfactants on current is shown in Figure 1. Using a borate buffer (10 mM, pH 9.0), at a voltage of 18 kV, the current flow was in the range of 52 - 75 μA. The figure shows that in 50 mM SDS, the current generated was 52 μA. The current proportionally increased as the percent of SC in SDS/SC mixed micellar system increased, then leveled off at 25:75% of SDS/SC.

Effect of Mixed Micelle on Migration Time Window

Sudan III is usually used in MEKC as an indicator to determine the migration time of the micelles, due to its hydrophobicity and high binding coefficient with the non-polar core of the micelles. Only one peak was detected as Sudan III in all five SDS/SC mixed micellar systems, which indicates that SDS/SC mixed micelles exhibited only one type of complex micelle, and not multiple micelles. This phenomenon was observed by others.[8-10] In 50 mM SDS buffer, the migration time of Sudan III (t_{mc}) was 16.13 min. The migration time increased from 16.13 to 19.28 min. as the percentage of SC in the buffer increased from 0-75%. However, the migration time dropped to 14.52 min. in 100% SC (Figure 2).

Bacci et al.[13] used an ionic hydrocarbon/non-ionic hydrocarbon mixtures of SDS/β-dodecyl maltoside (DM) to elucidate the theory of micellization of binary surfactant systems. The phenomenon is very similar to the SDS/SC mixed micellar system in the present study. In his model, in the presence of 0.2 M NaCl, when the mole fraction of DM increased from 0 to 75% of total surfactant, the micelle aggregation numbers also increased from about 110 in 100:0% SDS/DM to about 225 in 25:75% SDS/DM. When the mole fraction of DM changed to 100%, the aggregation numbers of micelles sharply decreased to about 100. The authors further indicated that when the mole fraction of DM in monomer changed from 0 to about 25%, the critical micelle concentration (CMC) of SDS in the SDS/DM mixed micellar system abruptly

decreased from about 6.4 to about 2 mM. A further increase in the mole fraction of DM to about 100%, the CMC of SDS surfactant was maintained at a flat level. Furthermore, as the mole fraction of DM in monomer increased from 0 to 25%, the mole fraction of DM in micelles was also sharply increased to >50% in SDS/DM mixed micellar system. When the mole fraction of DM in monomer continually increased to 100%, the mole fraction of DM in SDS/DM micelles system continually increased in sigmoid curve up to 100%.

If the theory is applicable to the SDS/SC mixed micelles, then the increase in migration time of micelles in SDS/SC mixed micellar system was due to the increase of aggregation numbers of micelles. This corresponds to the increase in size and total anionic charges on the surface of micelles, so the electrophoretic migration of micelles increased, and the migration time of micelle to the detector window also increased. The pure SC micelle, which tends to have lower aggregation number with smaller number of negative charges on the micelles, will results in slower electrophoretic migration, however, the dominant electroosmotic flow (EOF) makes the SC micelles to migrate faster than SDS because they are smaller.

Methanol, which is not retained in the micelle, is used for the determination of the migration time of the aqueous phase. It was observed that the SDS/SC mixed micellar systems, the migration time of methanol (t_o) slightly increased as the percentage of SC in SDS/SC mixed micellar systems increased.

It was suggested by Terabe[14] that t_o/t_{mc} value can be used for measurement of the migration time window of compounds in MEKC. For convenience, the t_o/t_{mc} value was transformed as t_{mc}/t_o value in later publications. The t_{mc}/t_o of pure SC and SDS micelles are 2.43 and 3.23, respectively. When the percent of SC in SDS/SC mixed micellar systems increased, the migration time window (t_{mc}/t_o) was proportionally increased and reached a peak value of 3.69, at 50:50% of SDS/SC. A further increase in percent of SC to 25:75, slightly decreased the migration time window (t_{mc}/t_o) to 3.57. The percent of SDS:SC at 50:50 produced an optimum migration time window, which is 50% higher than SC alone, and 15% higher than SDS alone.

Migration of Polycyclic Aromatic Hydrocarbons (PAHs) in SDS/SC Mixed Micelles

The pattern of migration times of fluoranthene in SDS/SC mixed micellar systems was very similar to the migration time of the tracer (Sudan III) *run in* SDS/SC MEKC. Because of its hydrophobic property, fluoranthene spent about

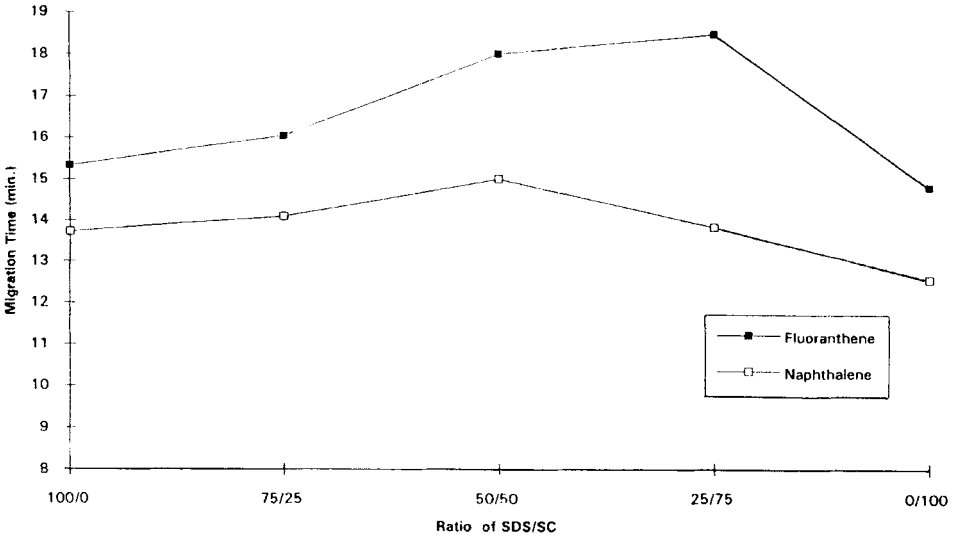

Figure 3. Effect of ratio of SDS/SC on the migration times of fuoranthene and naphthalene in MEKC. Experimental conditions as in Figure 1.

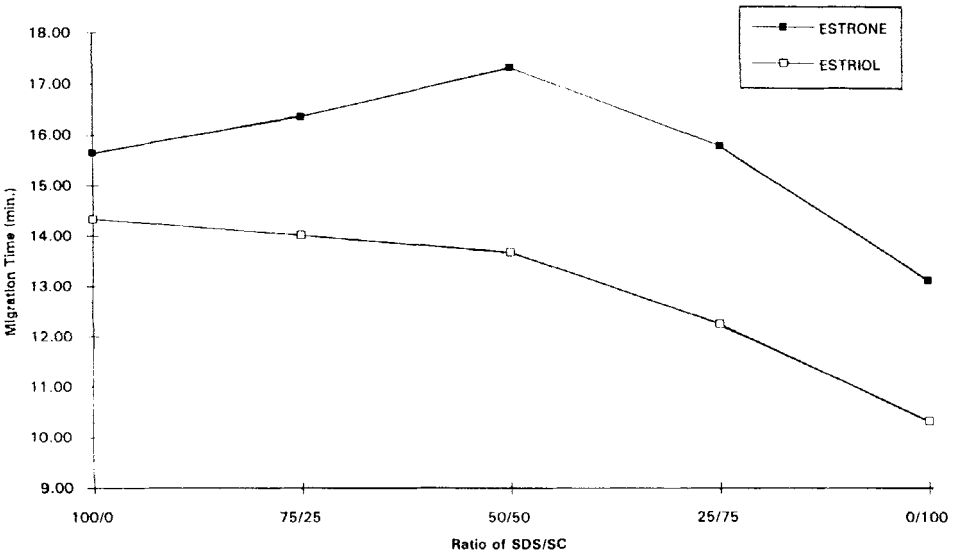

Figure 4. Effect of ratio of SDS/SC on the migration times of estrone and estriol in MEKC. Experimental conditions as in Figure 1.

95% of migration time in association with the micelles during the process of separation (Figure 3). This can be attributed to the high binding coefficient of this compound to the micellar entity.[15,16] Same phenomenon was observed for the naphthalene, which has two benzene rings, its binding coefficient to the SDS micelles was much smaller than fluoranthene, therefore, it eluted from the MEKC at a shorter time.

Migration of Estrogens in SDS/SC Mixed Micelles

The pattern of migration times of estrone in SDS/SC mixed micellar systems was similar to that of fluoranthene. Estrone spent about 82-97% of migration time associated with the micelles in the process of separation. Estriol is structurally similar to estrone, but more polar; this makes the binding coefficient of estriol in the SDS/SC mixed micellar systems less than that of estrone, which resulted in shorter migration times (Figure 4). Chan et al.,[17] in a study of separation of estrogens by MEKC, found that the migration time of estrone was longer than estriol under the same experimental condition, using a buffer system containing 50 mM SDS at pH 9.2.

Migration of Dansylated Amino Acids Containing Negatively Charged R Groups in SDS/SC Mixed Micelles

Dansylated aspartic acid, glutamic acid, and cysteic acid (CYA), at pH 9.0 are negatively charged.[18] In the pure SDS buffer solution, the negatively charged heads of SDS faced outside of the aqueous phase and the non-polar tails extended inside of the micellar spheres. As the negatively charged amino acids come in contact with the negatively charged surfaces of SDS, the electrostatic repulsion forces cause these amino acids to decrease their binding to the micelle aggregates. The result is that the amino acids eluted from the CE in a short time.

As the percentage of SC in the buffer increased and that SDS decreased to 75, 50, 25, and 0%, the electrostatic repulsion forces of SDS were proportionally decreased, but the binding forces of the hydrogen bonding, hydrophobic Van der Waals forces, and electrostatic attraction forces[19] increased, so the migration times of these acids increased proportionally (Figure 5). However, at 75% and 100% SC, the migration times of aspartic acid and glutamic acid increased abruptly. In all five SDS/SC mixed micellar systems, glutamic acid migrated faster than aspartic acid.

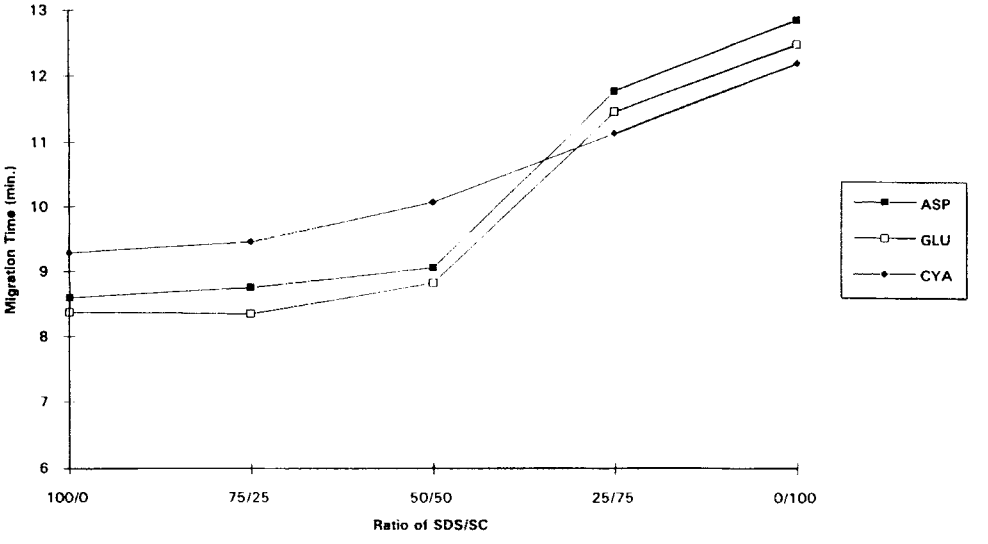

Figure 5. Effect of ratio of SDS/SC on the migration times of dansylated asparatic acid, glutamic acid, and cysteic acid (CYA) in MEKC. Experimental conditions as in Figure 1.

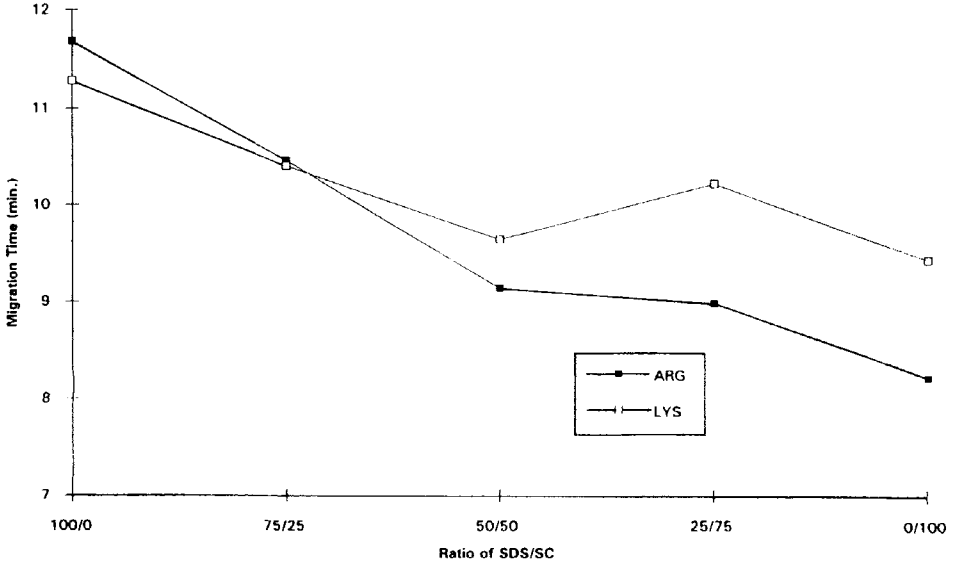

Figure 6. Effect of ratio of SDS/SC on the migration times of dansylated arginine and lysine in MEKC. Experimental conditions as in Figure 1.

The pattern of migration time of cysteic acid in SDS/SC five mixed micellar systems was similar to aspartic acid and glutamic acid. The migration time of cysteic acid proportionally increased with the increase in percentage of SC in SDS/SC mixed micellar system.

Migration of Dansylated Amino Acids with Positively Charged R-Group in SDS/SC Mixed Micelles

Dansylated arginine and lysine are basic amino acids. At pH 9.0, they are positively charged. In borate buffer solution containing pure SDS, the positively charged arginine and lysine are attracted to the negatively charged SDS aggregates, and are retained on the micelles surfaces, which resulted in longer migration time than the negatively charged amino acids. As the percent of SDS in SDS/SC mix micellar systems decreased to 75, 50, 25, and to 0%, the total negative charges of the mixed micelles also decreased, and the electrostatic binding forces to arginine and lysine were also correspondingly decreased, that resulted in a decrease of the migration time (Figure 6).

The migration time of arginine in pure SDS micelles was larger than that of lysine, but at SDS/SC (75:25%), their migration times became very close. As the percent of SDS in SDS/SC mix micellar systems decreased to 50, 25, and 0%, the migration time of arginine became smaller than that of lysine. This change in elution order was probably due to the change of binding coefficients of the solutes to the pseudo-stationary phase.

Migration of Dansylated Amino Acids with Aromatic R-Groups in SDS/SC Mixed Micelles

The pattern of migration time of di-dansylated tyrosine was similar to that of Sudan III in SDS/SC five mixed micellar systems, because didansylated tyrosine is relatively non-polar. When it is dissolved in the micelle, it would take long time to elute from the MEKC. This phenomenon was reported by Chrambach and Hjelmeland[20] in a study dealing with the CE separation of 20 dansylated amino acids in a micellar buffer made of 100 mM borate, pH 9.0, containing 50 mM SDS. The migration times of dansylated phenylalanine and tryptophan in all the SDS/SC mixed micellar systems were very close to each other (Figure 7). The use of SDS and SC mixed micellar system without the addition of modifiers were not sufficient to achieve good resolution and selectivity of phenylalanine and tryptophan under the present experimental conditions. The migration times of phenylalanine in SDS/SC five mixed micellar systems were slightly shorter than those of tryptophan (Figure 7).

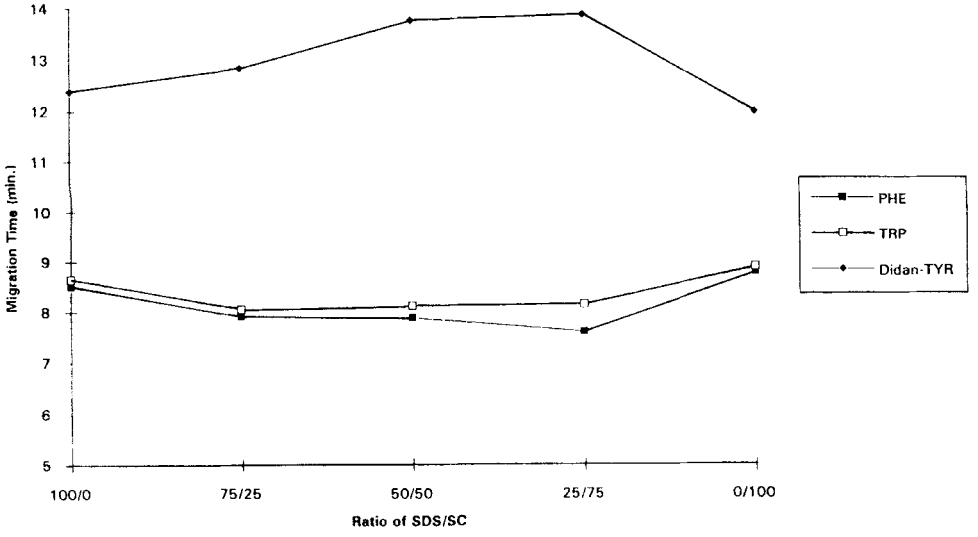

Figure 7. Effect of ratio of SDS/SC on the migration times of dansylated phenylalanine, tryptophan, and di-dansylated tyrosine in MEKC. Experimental conditions as in Figure 1.

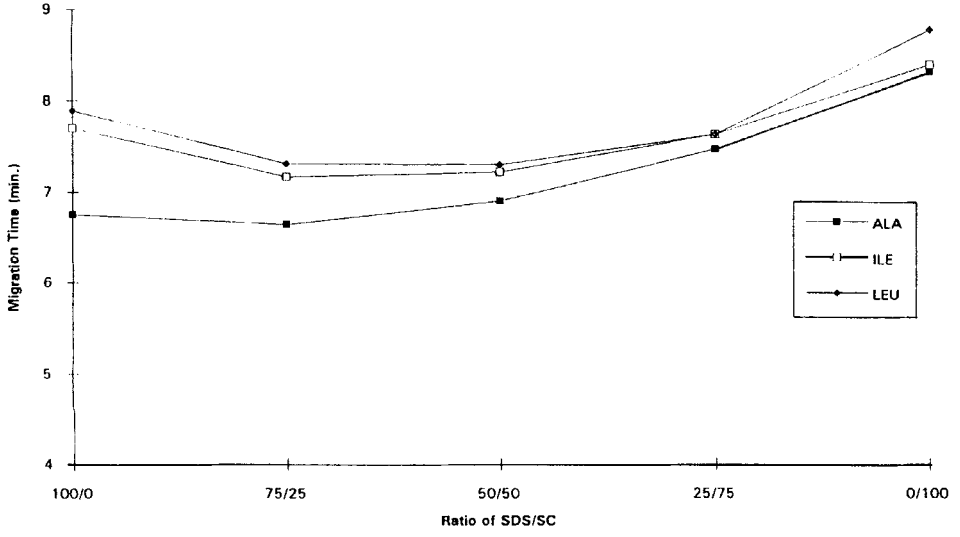

Figure 8. Effect of ratio of SDS/SC on the migration times of dansylated alanine, leucine, and isoleucine in MEKC. Experimental conditions as in Figure 1.

Migration of Damsylated Amino Acids with Aliphatic R-Group in SDS/SC Mixed Micelles

Dansylated alanine, leucine and isoleucine exhibited similar migration times in the five mixed micellar systems with slight variation at 100% SC (Figure 8).

Migration of Dansylated Amino Acids with Polar but Uncharged R-Group in SDS/SC Mixed Micelles

Dansylated glutamine and serine in SDS 100% micellar system migrated close to each other. but in the other four ratios of SDS/SC mixed micellar systems, the two compounds were separated (Figure 9). The migration times of glutamine were smaller than those of serine in SDS/SC five mixed micellar system.

Migration of Dansylated Glycine and Proline in SDS/SC Mixed Micelles

Dansylated glycine and proline were resolved in 100% SDS, 100% SC, and at 25:75% SDS/SC. However, the migration times of these two compounds in 75:25 and 50:50% were so close that there was no resolution between them (Figure 10).

Overall, the results indicated that the use of SDS and SC in five mixed micellar system were not sufficient in extending the migration time window to allow a satisfactory separation of a mixture of all amino acids used in this study. No optimization of such a separation was attempted, because this was not the objective of this research.

CONCLUSION

In this SDS/SC buffer system MECK, the migration time window, (t_{mc}/t_o), at 100% SC was found to be 2.43. The addition of SDS to the SC at the SDS/SC ratio of 50:50, the migration time window (t_{mc}/t_o), increased to 3.69, which is 50% higher than using pure SC and 15% higher than using pure SDS. The increase in migration time window in SDS/SC mixed micellar systems, is due to an increase in aggregation numbers of the micelles. The results show that the physical properties of the micelles, as well as the test solutes; hydrophobic, polar, positively or negatively charged influenced the migration times.

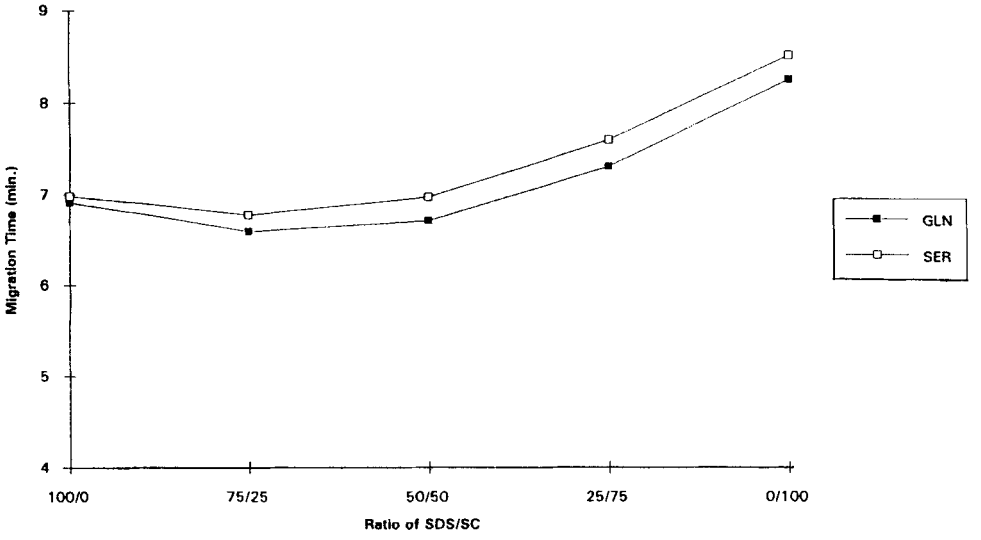

Figure 9. Effect of ratio of SDS/SC on the migration times of danslyated glutamine and serine in MEKC. Experimental conditions as in Figure 1.

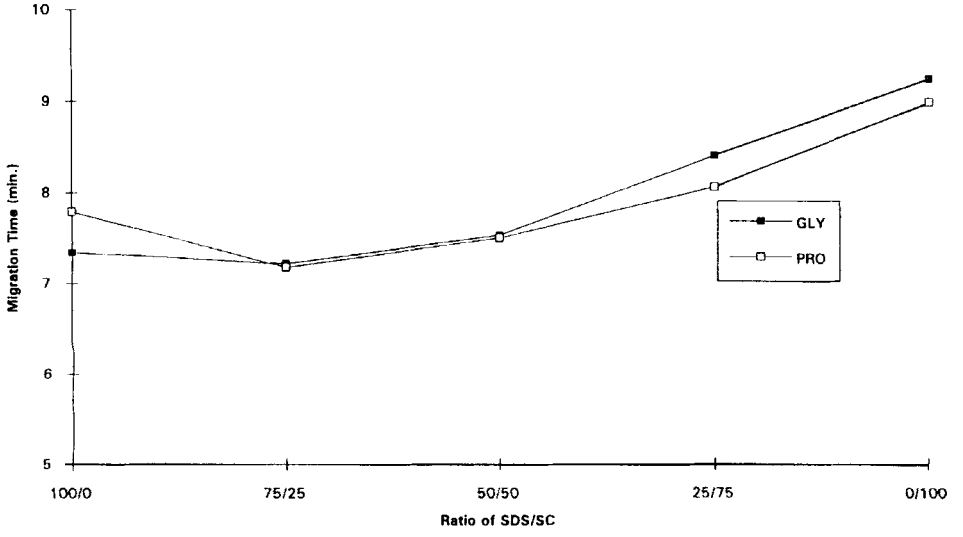

Figure 10. Effect of ratio of SDS/SC on the migration times of dansylated glycine and proline in MEKC. Experimental conditions as in Figure 1.

This can be explained by the ability of the solutes to interact with the micellar system or be repelled by it. Although most amino acids are negatively charged at pH 9, they showed different migration patterns in the five different mixed micelles due to the chemical/physical properties of their R-group. For example, the migration times of the positively charged amino acids decreased with an increase in SC concentration while the negatively charged amino acids eluted faster. Also, the hydrophobic PAHs, which are expected to bind to SDS eluted faster when the concentration of SC was increased to 100%.

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