Characterization of mixed micellar pseudostationary phases in electrokinetic chromatography using linear solvation energy relationships

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Abstract

The influence of mixed micellar systems on retention and selectivity in micellar electrokinetic chromatography is examined using linear solvation energy relationships (LSER). Systems that were investigated include mixed bile salts [sodium deoxycholate (SDC) and sodium cholate (SC)] and mixed sodium dodecyl sulfate (SDS)–bile salt systems (e.g., SDS–SC and SDS–SDC). The retention behavior in individual and mixed micellar systems is primarily determined by size and hydrogen bond acceptor strengths of solutes. Through a comparative study of the LSER coefficients in the individual and mixed micellar systems, it was concluded that hydrogen bonding interactions have a significant effect on selectivity of these pseudostationary phases in electrokinetic chromatography. The interactive properties of the mixed micelles are different from the constituent individual micelles, however, the overall characteristics are closer to one of the bile salt micelles in the mixture even at the equimolar compositions. © 1998 Elsevier Science B.V.

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1. Introduction

Separation of uncharged solutes in micellar electrokinetic chromatography (MEKC) is due to their differential interactions with a charged pseudostationary phase [1,2]. The composition of the micellar solution, especially the type of surfactant, has a great influence on the overall retention behavior and separation. Over the past decade, a variety of micelle forming surfactants and polymeric phases have been successfully applied in MEKC separations. Resolution can also be enhanced through the proper adjustment of the composition of micellar solution by including various modifiers such as organic solvents, urea, cyclodextrins and glucose [3,4].

The availability of a wide variety of pseudostationary phases with different selectivities is quite advantageous in method development. The large number of choices, however, would make the process of selecting the optimum type and composition of pseudostationary phase difficult. The problem is particularly pronounced for the separation of complex mixtures where operating under optimum conditions is crucial. In order to facilitate optimization of buffer composition in MEKC, one should achieve a better understanding of the exact nature of solute interactions with pseudostationary phase that control retention and selectivity. The first effort to address this issue was reported by Yang and Khaledi through

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the use of linear solvation energy relationships (LSERs) [5,6]. The LSER methodology was first developed by Kamlet, Taft and coworkers and has been applied to describe ''solvation effects'' in many physico-chemical and biological systems, including solute distribution in water–octanol [7] and water–micelle [8,9] systems as well as chromatographic retention [10,11]. The LSER models allow quantitative description of retention in MEKC in terms of underlying interactions between solutes and micellar systems as:

$$\log k' = \log k_o + mV_1/100 + b\beta + a\alpha + s\pi^* \qquad (1)$$

where V is the intrinsic molar volume of the solute, π^* is the measure of dipolarity–polarizability, β is solute hydrogen bond acceptor strength (basicity), and α is hydrogen bond donor strength (acidity) of solute. The $mV/100$ term is related to hydrophobic interaction as it represents an unfavorable energy term for the formation of a properly sized cavity in the solvent system in order to accommodate solute. The $b\beta$ and $a\alpha$ represent types A and B hydrogen bonding, while $s\pi^*$ is a measure of dipolar interactions in the system. The coefficients m, s, b, and a in the LSER equation are related to the interactive properties of the micellar solution. Through a comparative study of the four coefficients m, b, a and s for different pseudostationary phases, one can categorize the interactive characteristics and subsequently chemical selectivity of MEKC systems. The details of the LSER results for various surfactants and their application for rationalizing retention and selectivity in MEKC have been published previously [5,6,12,13]. Recently, other workers have also reported the LSER modeling of MEKC retention for different systems [14,15].

In this work, LSER models are applied in order to characterize the nature of solute interactions with mixed micellar systems of sodium dodecyl sulfate (SDS) and bile salts. SDS has been the most commonly used surfactant in MEKC. As an alternative, bile salts have been utilized in situations where SDS micelles do not provide adequate resolution. In general, the elution patterns and selectivity for the SDS and bile salts micelles are quite different. Mixed micelles influence MEKC separations through their impact on retention, selectivity

and the size of the elution window [16–20]. Mixtures of bile salts and anionic alkylated surfactants (including SDS) have been quite effective in improving MEKC separation of closely related compounds [16–19].

2. Experimental

2.1. Apparatus

All data for LSER models was collected on a laboratory-built CE system that comprised a 0–30 kV high voltage power supply (Series EH, Glassman High Voltage, Whitehouse Station, NJ, USA), a variable wavelength UV–Vis detector (Model 500, SSI, State College, PA, USA) operating at 254 nm, and 50 μm I.D.×370 μm O.D. fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA). The total length of the capillary was 62 cm and the effective length was 50 cm. An applied voltage of +20 kV was used. Electropherograms were collected with an electronic integrator (Hewlett-Packard, Avondale, PA, USA).

The electropherograms for the two test mixtures were collected on a Beckman P/ACE system 5500 (Beckman, Palo Alto, CA, USA) with a 57 cm long fused silica capillary. The effective length of the capillary was 50 cm. All analyses were performed with UV detection at 254 nm. The applied voltage was +20 kV. The temperature was maintained at 25°C.

2.2. Reagents

SDS (99% pure) was purchased from Sigma (St. Louis, MO, USA) and used as received. SC and SDC were purchased from Aldrich (Milwaukee, WI, USA) and Sigma. All test solutes were purchased from Aldrich. Buffer solutions were prepared by dissolving the required amount of surfactant in doubly distilled deionized water and were filtered through a 0.45 μm polypropylene membrane filter (SRI, Eatontown, NJ, USA). The SDS, SC and mixed SDS–SC solutions were buffered at pH 7.0 with 50 m*M* phosphate buffer. For SDC solutions the pH was kept at 9.0 for solubility reasons using 50 m*M* phosphate–borate buffer. The capillary was rinsed

with buffer solution between each run for 2 min and was rinsed with methanol, sodium hydroxide (1 M) and water between buffers. The migration time of an unretained solute (t_{eo}) was measured from the time of injection to the first deviation of the baseline for the solvent peak, methanol. The t_{mc} marker was n-dodecanophenone.

3. Results and discussion

In Table 1, the LSER coefficients for SDS, SC, SDC, mixed micelles of the two bile salts (SC–SDC), and mixed micelles of SDS with the two bile salts micelles are listed. A group of 60 aromatic solutes was used for deriving the LSER models as in a previous study [6]. The test solutes can be categorized according to their hydrogen bonding solvatochromic parameters (α and β) into three subgroups of hydrogen bond acceptors (HBAs), hydrogen bond donors (HBDs), and nonhydrogen bond donors (NHBs). Note that SDC is not soluble at pH 7.0, thus all solutions that include SDC were buffered at pH 9.0. At this pH, some of the HBD phenols are partially ionized. The equation used to calculate the retention factors, k', of ionized solutes includes a correction term for the electrophoretic mobilities in the bulk aqueous solvent (i.e in the absence of micelles) as described earlier [21,22]. Nevertheless,

pH influences the LSER coefficients as can be seen for the SC results at pH values of 7.0 and 9.0. The LSER results for 40 mM SDS was reported earlier [6] and are also used in this study. It is important to note that surfactant concentration has no effect on the LSER results. Thus, the coefficients for the SDS can be compared to the other micellar systems in spite of the different total surfactant concentrations.

The LSER coefficients of m and b have the largest values that show size and hydrogen bond acceptor strengths of solutes are the two main contributing factors to retention (Table 1). Bulkier molecules are retained longer (due to large positive m-coefficients) while stronger HBA solutes interact less with the micelles and have shorter retention (due to the negative b coefficient). These results are similar to those observed for other pseudostationary phases in MEKC — with the exception of lithium perfluorooctanesulfonate (LiPFOS) micelles [6]. The m coefficients are very similar for the individual, and the mixed micellar systems that indicate selectivity differences between these pseudostationary phases are not due to the size effects. It has been determined previously that the main source of selectivity variations between SDS and SC is due to hydrogen bonding effects [6]. The b-coefficient is related to the hydrogen bonding donor strength of the pseudostationary phases. The larger b value (less negative) means that SDS micelles provide stronger HBD sites

Table 1
The LSER coefficients for SDS, SC, SDC, mixed SDS–SC, and mixed SDS–SDC micellar pseudostationary phases in MEKC [Eq. (1)]

Pseudostationary phase	$\log k_o$	m	s	b	a	r	S.E.
40 mM SDS pH=7.0	−1.49	3.95 (0.39)	−0.26[a] (0.31)	−1.80 (0.31)	−0.18 (0.15)	0.955	0.156
30 mM SDS–30 mM SC, pH=7.0	−1.53	3.88 (0.37)	−0.20[a] (0.28)	−2.57 (0.30)	0.23 (0.16)	0.965	0.143
60 mM SC pH=7.0	−1.62	3.89 (0.39)	−0.27[a] (0.31)	−2.88 (0.31)	0.23 (0.15)	0.968	0.144
60 mM SC pH=9.0	−1.55	3.65 (0.44)	−0.25 (0.33)	−3.11 (0.36)	0.84 (0.19)	0.954	0.17
30 mM SDS–30 mM SDC, pH=9.0	−1.56	3.94 (0.18)	−0.24 (0.13)	−2.39 (0.14)	0.14 (0.08)	0.968	0.136
60 mM SDC pH=9.0	−1.51	4.00 (0.38)	−0.35 (0.28)	−2.77 (0.30)	0.07[a] (0.14)	0.969	0.115
30 mM SC–30 mM SDC, pH=9.0	−1.54	3.88 (0.37)	−0.31 (0.28)	−2.81 (0.30)	0.17 (0.16)	0.968	0.14

A set of 60 aromatic test solutes was used as in Ref. [6] (n=60), r=correlation coefficient. S.E. is the standard error of estimated $\log k'$. The numbers in parentheses represent the 95% confidence intervals for the coefficients.
[a] Values are not significant at the 95% confidence level according to t-test results.
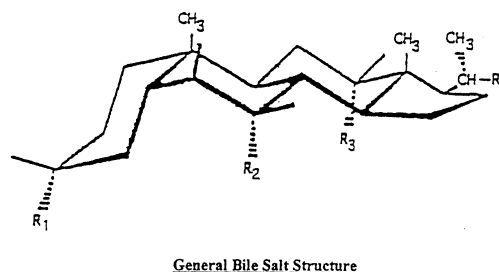
General Bile Salt Structure

Bile Salt (Sodium)	R_1	R_2	R_3	R
Cholate	-OH	-OH	-OH	$-CH_2CH_2COO^-$
Deoxycholate	-OH	-H	-OH	$-CH_2CH_2COO^-$

Fig. 1. Structures of bile salt surfactants.

than the two bile salts and the mixed micelles. The bile salts and their mixed micelles have similar HBD strengths. On the other hand, the larger *a*-coefficients indicate that the bile salts and the mixed micellar systems are stronger hydrogen bond acceptors than SDS micelles. Comparing the two bile salts and their mixture, the SC micelles are the strongest HBA. This might be due to the extra hydroxyl group on the SC backbone (see Fig. 1). In spite of their high structural similarity, SC and SDC exhibited different selectivities for the MEKC separation of a group of corticosteroids [16,17]. As shown in Fig. 2, the variations in the coefficients *a* and *b* are mirror images of one another. As *b* becomes more negative,
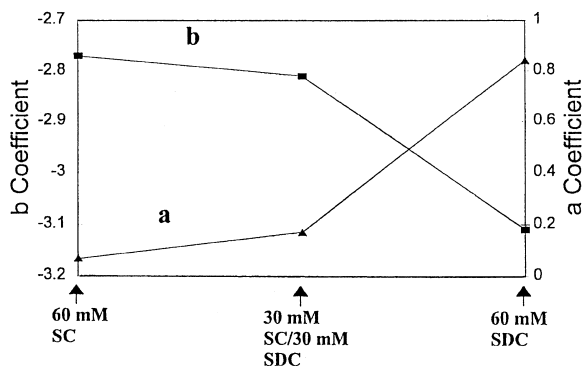
indicating a decrease in HBD ability, *a* becomes more positive, indicating an increase in HBA strength. Similar trends were observed for the SDS–SC and SDS–SDC systems.

Another way of investigating solute–micelle interaction is by calculating the change in free energy of transfer, $\Delta\Delta G$, of functional groups from the bulk aqueous solvent to the micellar pseudostationary phase. The $\Delta\Delta G$ values for various substituents can be determined from the functional group selectivity, τ, as $\Delta\Delta G = -RT\ln(\tau)$ where τ is the ratio of the migration factor, k' of a substituted benzene (Ph–R) over the migration factor of benzene (Ph–H) (Table 2). Negative $\Delta\Delta G$ values mean favorable interactions between the functional groups and the micelles. For such cases, the addition of the functional group to the parent compound (benzene) leads to an increase in the interaction with the micelles. A positive $\Delta\Delta G$, on the other hand, indicates that the addition of a functional group to benzene leads to a decrease in the interaction with the micelle. Therefore, the larger negative $\Delta\Delta G$ shows a more favorable interaction, and larger positive $\Delta\Delta G$ indicates that the interactions between the substituted benzenes and micelles are less favorable than that for the parent benzene. As shown in Table 2, the HBA groups favor the SDS micelles over SC and SDS–SC systems. In addition, the $\Delta\Delta G$ values and trends in the mixed SC–SDS system are closer to those in the SC micelles. This reinforces the LSER results that suggest mixed SDS–bile salt systems have properties that are more similar to bile salt systems than SDS systems.

The similarity of migration patterns between the SDS–SC and the SC micelles can also be seen in Fig. 3a with $r = 0.991$. A lower correlation coefficient ($r = 0.951$) was observed between retention in the mixed micelle and that in the SDS micelles (Fig. 3b). The lowest correlation coefficient was observed between retention in SDS and SC micellar systems with $r = 0.933$ (Fig. 3c). The lines in Fig. 3a–c show the relationships between retention of the NHB compounds only. Overall, high correlations exist between retention behavior with different pseudostationary phases in MEKC. This is due to the predominant effect of hydrophobic interaction on retention in MEKC that is represented by the large, positive *m* coefficient in the LSER model.

Fig. 2. Comparison between the LSER *a* coefficients (filled triangles) and *b* coefficients (filled squares) in 60 m*M* SC, mixed 30 m*M* SC–30 m*M* SDC, and 60 m*M* SDC micelles.

Table 2
Free energy of transfer for functional groups in SDS, SC and SDS/SC pseudostationary phases [$\Delta\Delta G$ (KJ/mol)]: $\Delta\Delta G = -RT\ln \tau$ where τ is functional group selectivity

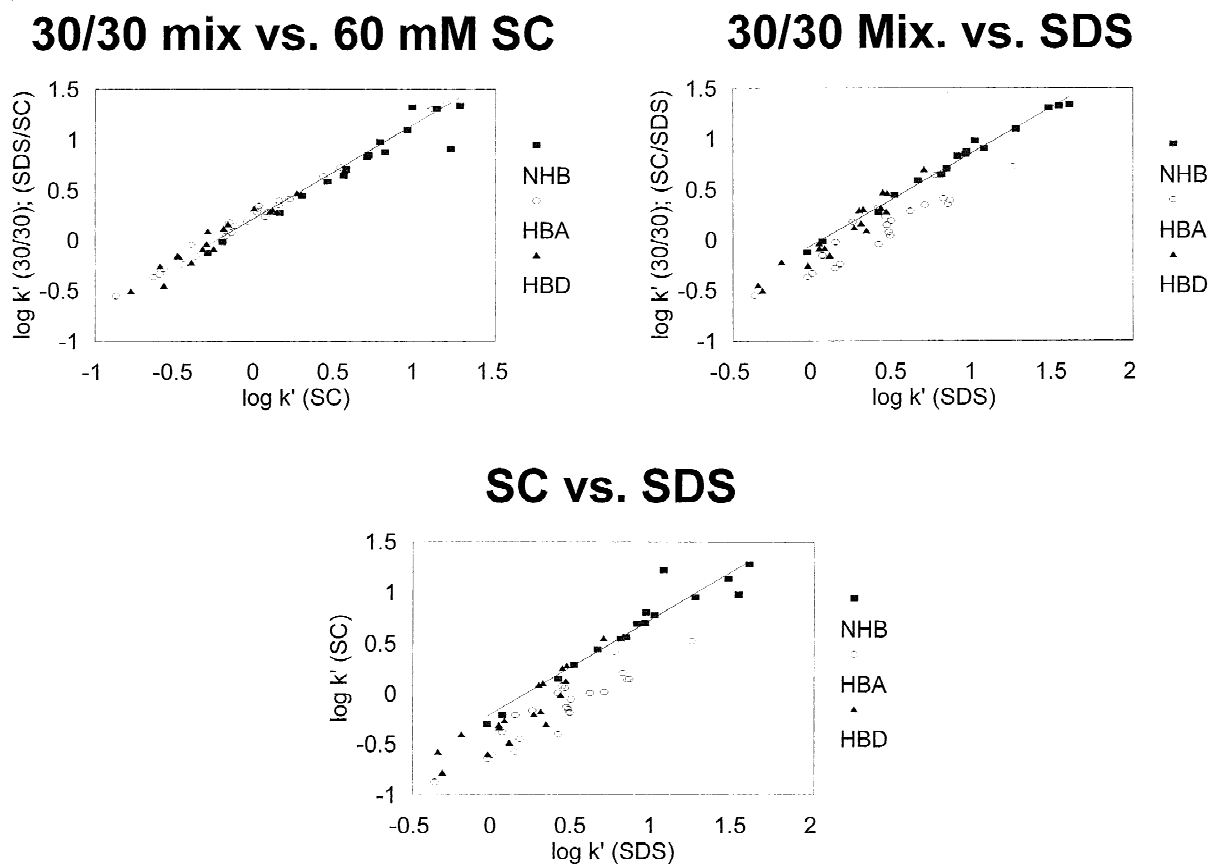| Functional group	40 mM SDS	30 mM SDS–30 mM SC	60 mM SC
–CHO	−0.013	1.40	1.92
–CN	−0.17	1.21	1.72
–NO$_2$	−0.54	0.19	0.44
–O$_2$CCH$_3$	−0.98	0.91	1.56
–COCH$_3$	−1.17	0.69	0.86
–COCH$_2$CH$_3$	−2.95	−0.95	−0.65
–COCH$_2$CH$_2$CH$_3$	−5.00	−2.74	−2.53
–COCH$_2$CH$_2$CH$_2$CH$_3$	−7.30	−4.85	−4.72
–OCH$_3$	−1.01	−0.56	−0.52
–OCH$_2$CH$_3$	−2.77	−2.05	−2.05
–CO$_2$CH$_3$	−2.90	−1.44	−0.84
–CO$_2$CH$_2$CH$_3$	−5.07	−2.96	−2.55

Fig. 3. Relationships between retention in different micellar pseudostationary phases. (top right) log k' (30 mM SC–30 mM SDS) vs. log k' (40 mM SDS); (top left) log k' (30 mM SC–30 mM SDS) vs. log k' (40 mM SC); (bottom) log k' (60 mM SC) vs. log k' (40 mM SDS).

In spite of the high correlations between $\log k'$, there exist large selectivity differences between these systems, mainly as a result of hydrogen bonding interactions. For example, in the mixed micelle vs. SDS and SC vs. SC plots, (Fig. 3), one can recognize a trend in the grouping of solutes according to their hydrogen bonding characteristics. In general, the HBA solutes are grouped below the HBD compounds. This is in accordance with the LSER characterization of SC and the mixed micelles as being stronger HBA than SDS. It should be noted that the grouping is not strictly according to the hydrogen bonding as the solutes have different sizes and dipolarity–polarizability. In addition, all of the HBD solutes can also act as HBA. In fact, some of the aromatic alcohols have stronger acceptor tendency (i.e. $\beta > \alpha$). These factors would cause overlaps between the subgroups, nevertheless, clustering of most of the HBA and HBD solute is quite evident.

Fig. 4 illustrates the different elution patterns of a test mixture of four aromatic solutes in the SDS, SC, and a mixed SDS–SC system. The four test solutes were selected according to their solvatochromic properties (Table 3): 4-chlorophenol is a HBD, toluene a NHB, 4-chloronitrobenzene a weak HBA, and propiophenone a strong HBA compound.

As shown in Fig. 4a, with the HBD micelles of SDS, the HBD solute, 4-chlorophenol, elutes first while the two HBA (peaks 3 and 4) elute last. This trend is the opposite to that observed for the HBA systems of SDS–SC and SC (Fig. 4b,c). In order to rationalize the exact order of elution for all peaks, one should consider the effects of all types of interactions as modeled by LSER. For example, with the SC micelles, toluene elutes after 4-chlorophenol, in spite of the smaller size and lack of a HBD functional group ($\alpha = 0$) (Table 3). One would expect the opposite behavior considering the strong HBD ability of the phenol and the importance of solute size in determining retention. Note, however, that 4-chlorophenol is a stronger HBA (larger β in Table 3) and more polar–polarizable solute (larger π^*) than toluene. These two factors offset the effects of size and type-B hydrogen bonding, i.e. smaller retention for larger β and π^* due to the negative b and s coefficients in the LSER model for SC. As shown in Fig. 4c, the selectivity differences between SC and mixed SDS–SC systems can be

substantial. It is remarkable that the elution order in the mixed SDC/SC system and SC (Fig. 4c) differ in spite of the very high correlation between retention in the two systems (Fig. 3).

Similar results were also observed for the SDC and mixed micelles of SDS–SDC. This can be seen in the chromatograms of another test mixture shown in Fig. 5a–c. The solvatochromic parameter values and intrinsic volume for this group of test mixtures are listed in Table 4. Again, with the HBD–SDS micelles, the HBD solute, 4-iodophenol, elutes first, while the two HBA compounds elute last. The trends are the opposite for the mixed SDS–SDC and SDC systems. Selectivity differences are also observed for the latter two pseudostationary phases.

Fig. 6a–d illustrates the variations in retention ($\log k'$) as a function of the mole fraction of SDC in a mixed micellar system of SDS–SDC with a total concentration of 100 mM for solutes with hydrogen bond functional groups (Fig. 6a), homologous series of alkyl aryl ketones (Fig. 6b), homologous series of nitroalkanes (Fig. 6c), and NHB solutes (Fig. 6d). As can be seen, the retention of all solutes (with the exception of resorcinol, see Fig. 6a) decreases with an increase in mole fraction of SDC in the mixed micelles. For longer retained solutes, retention levels off or begins to increase at X(SDC) = 0.80 for some of the longer retained solutes. The reason for this behavior is not totally clear. The source can simply be the uncertainties in measuring t_{mc} that lead to significant errors in determining large k' values.

Variations in retention with the composition of mixed micelles can be due to changes in the volume phase ratio as well as different distribution ratios of solutes between the bulk aqueous phase and the mixed micellar pseudostationary phase. Unfortunately, the information about the chromatographic phase ratios for these systems is not available. However, the variations in the phase ratio is the same for all solutes. Selectivity (or relative retention) between solutes is independent on phase ratio.

The same overall trends were observed for all solutes at another total surfactant concentration (75 mM). Fig. 6e shows the results for the hydrogen bonding solutes group that are nearly identical with those in Fig. 6a. This confirms the fact that surfactant concentration has little or no effect on selectivity of uncharged solutes.
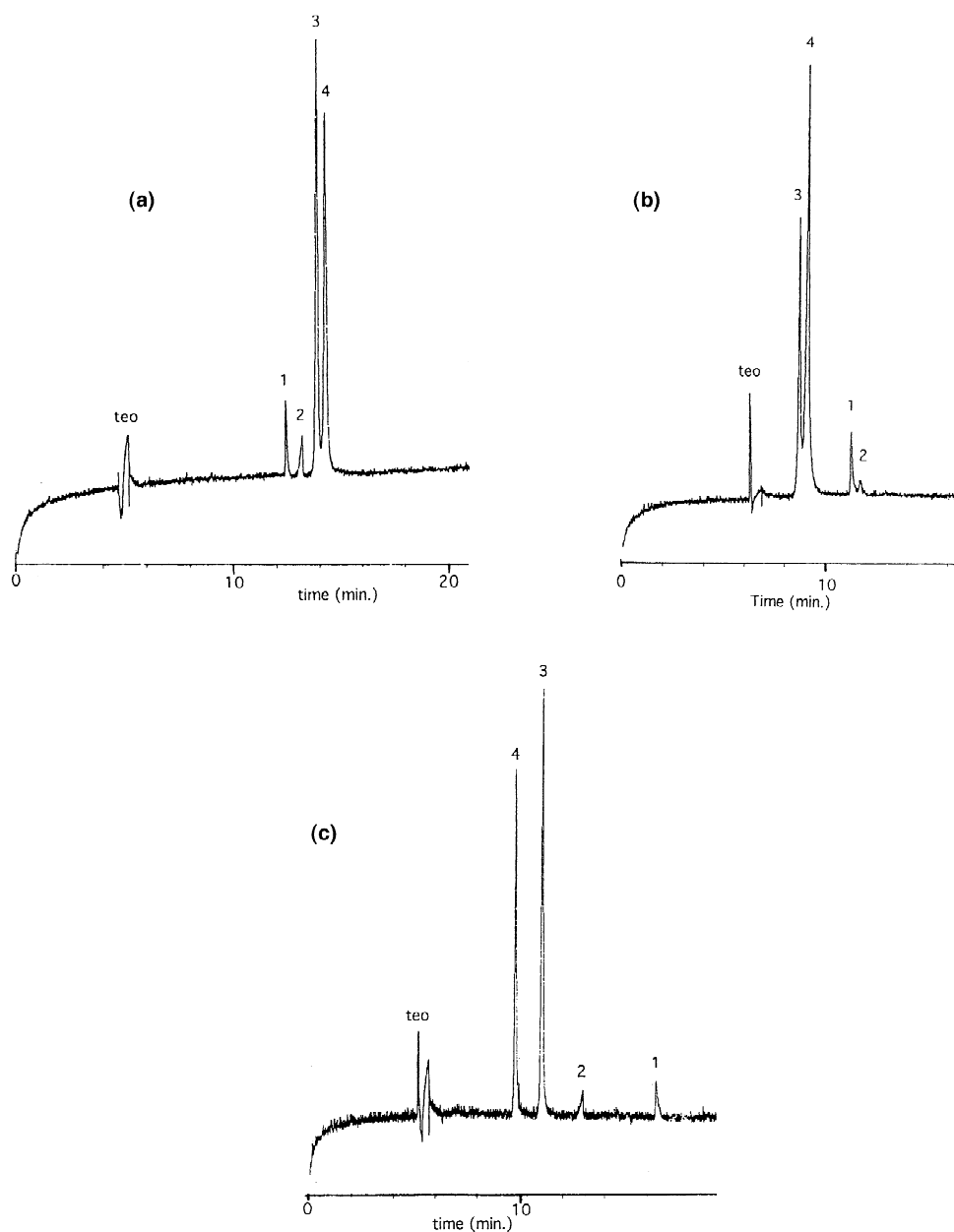
Fig. 4. Comparison elution patterns and selectivity in different micellar systems for a test mixture of four solutes using 50 m*M* phosphate buffer, pH 7.0 containing: (a) 60 m*M* SDS, (b) 60 m*M* SC, (c) 30 m*M* SDS and 30 m*M* SC. Other conditions given in Section 2. Peak identifications as in Table 3.

The rate of decrease in retention, however, varies for solutes with hydrogen bonding functional groups, while it is almost the same for NHB compounds, as well as the members of the two homologous series,

as evident by the parallel lines in Fig. 6b–d. The overall retention patterns can be rationalized based on the LSER characterization of these systems. The *m* coefficients for the SDS, SDC, and the mixed

Table 3
Properties for the test solutes in Fig. 4a–c

Peak[a]	Solutes	$V/100$	π^*	β	α	$\log P_{ow}$
1	4-Chlorophenol	0.626	0.72	0.23	0.67	2.35
2	Toluene	0.592	0.55	0.11	0	2.69
3	4-Chloronitrobenzene	0.721	1.01	0.26	0	2.41
4	4-Propiophenone	0.788	0.88	0.49	0	2.19

[a] Peak number in chromatograms in Fig. 4a–c.

micelles are nearly identical. As a result, the rate of change in retention for solutes with different sizes but similar or equal hydrogen bond donor–acceptor strengths would be constant, i.e. little or no change in selectivity among these solutes. This can be seen in Fig. 6b,c for the two homologous series of nitroalkanes and alkyl aryl ketones. Both classes carry a HBA functional group (nitro and carbonyl, respectively). Consequently, they have overall stronger interactions with the HBD–SDS micelles than with the HBA–SDC or mixed micelles. This contributes to the decrease in retention of the homologous compounds. However, since the difference between individual solutes within a series is size (the number of methylene groups), there is no significant variation in selectivity between any two solutes in the series (the lines are almost parallel). A similar trend is observed for the four NHB aromatic compounds, in Fig. 6d, that are actually weak hydrogen bond acceptors ($\beta = 0.07$–0.11). Thus, the selectivity change is almost negligible between these solutes. Large variations in selectivity were observed for solutes that carry hydrogen bond functional groups, as is evident by the lines with different slopes in Fig. 6a and Fig. 6e. The lines for certain solutes cross one another that indicate the elution order has changed with the composition of the mixed micellar phase. For a HBD solute such as phenol, the rate of decrease in retention is much smaller and remarkably, for resorcinol, retention even increases systematically as the mole ratio of the HBA micelles of SDC is increased. Note that all solutes that are categorized as HBD in accordance to their solvatochromic parameters also have measurable HBA properties (consider phenol for example with $\alpha = 0.61$ and $\beta = 0.33$). As a result, their retention behavior is a balance between the two types of hydrogen bonding interactions. On the one hand, they have a higher affinity for the micelles con-

taining HBA–SDC, on the other hand their HBA property results in less retention (larger negative b-coefficients for SDC and the mixed micelles). Fig. 7a,b clearly support the above statements as the selectivity (defined as the ratio of retention factors for two solutes, $\alpha = k'_2/k'_1$) remains nearly constant when both solutes belong to the NHB or are members of a homologous series, while great variations are observed even if only one of the two compounds has a hydrogen bond functional group.

Interestingly, the interactive (especially the hydrogen bonding) properties of the mixed micellar systems of SDS–bile salts are closer to the individual bile salts micelles than to the SDS micelles. The exact reasons are not known and depend on the structural and physico-chemical properties of the aggregates. In order to shed light onto the behavior of the mixed systems, one should first achieve a better understanding of the nature of the hydrogen bonding sites in individual micelles. Consider the SDS micelles that exhibit a fairly strong donor characteristic. Due to the lack of any HBD group on the SDS molecule, one can only point out the water molecules that are localized in the palisade and Stern layers of the SDS micelles as the HBD source. Apparently the water molecules that are localized within or at the surface of the micelles have different hydrogen bonding strengths than those in the bulk medium. Other workers have estimated the Kamlet–Taft solvatochromic parameter α (a measure of the hydrogen bond donor strength) for the SDS micelles to be around 0.6 and 1.0 [9,23]. Bulk solvent water has an α value of 1.17.

The structures and aggregation properties of the bile salts are very different from SDS micelles. They form smaller primary micelles with aggregation numbers between two and ten. At higher bile salts concentrations, secondary micelles with much larger aggregation numbers might be formed. The aggrega-
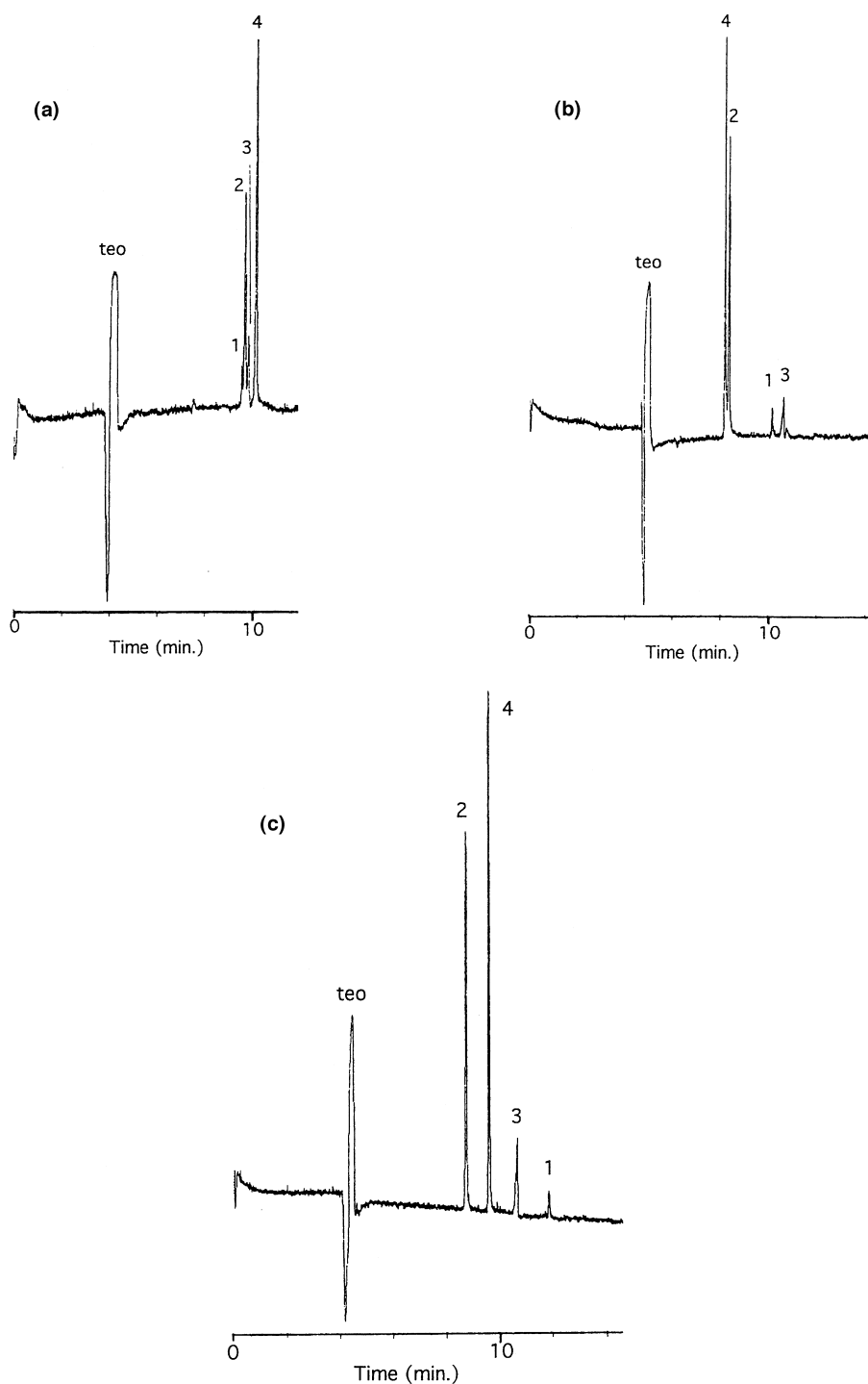
Fig. 5. Comparison of elution patterns and selectivity in different micellar systems for a test mixture of four solutes using 50 m*M* phosphate–borate buffer, pH 9.0 containing: (a) 60 m*M* SDS, (b) 60 m*M* SDC (c) 30 m*M* SDS and 30 m*M* SDC. Other conditions given in Section 2. Peak identifications as in Table 4.

Table 4
Properties for the test solutes in Fig. 5a–c

Peak[a]	Solutes	$V/100$	π^*	β	α	$\log P_{ow}$
1	4-Iodophenol	0.716	0.81	0.35	0.71	2.91
2	4-Bromonitrobenzene	0.764	1.01	0.26	0	2.55
3	Ethylbenzene	0.668	0.53	0.12	0	3.15
4	4-Chloroacetophenone	0.780	0.90	0.45	0.06	2.35

[a] Peak number in chromatograms in Fig. 5a–c.

tion process of bile salts has been a controversial matter [24–27]. One theory assumes that aggregation is primarily due to interaction of the hydrophobic backbones of the bile salts molecules (back-to-back model), leaving the polar hydroxyl functional groups in contact with water [25,26]. The other model (face-to-face model), proposes a dimer formation at pre-micellar concentration as a result of hydrogen bonding interaction between the hydroxyl groups of two bile salts molecules. As mentioned above, the LSER results indicate that the two bile salts micelles behave as HBA. The hydroxyl functional groups on the steroidal backbone of the bile salts micelles are probably the main HBA sites. Thus, the LSER results seem to be in greater agreement with the back-to-back model that leaves the hydroxyl groups available for interaction with solutes.

Due to the nonideal behavior of the SDS and bile salts mixtures, one can anticipate that several types of aggregates with different compositions and structures coexist in the solution – with one being the predominant form depending upon the mole fractions of the two constituent surfactants. Based on the NMR self-diffusion and relaxation studies of mixtures of SDS and SC, Wiedmer et al. concluded that the fraction of the SDS molecules in the monomeric form decreases upon the addition of SC [19]. This seems to be due to the initial solubilization of SC molecules in the SDS micelles that results in the formation of larger mixed micelles. One can envision that, at low concentrations, the bile salts molecules "partition" into the SDS micelles with their hydrophobic moieties facing towards the hydrophobic interior of the roughly spherical SDS micelles and their polar surface oriented toward the outside. Under such a circumstance, the localized HBD water molecules in the SDS micelles are engaged with the

HBA hydroxyl groups of the bile salts, making them less available for interaction with the solutes. This explains the large decrease in the *b* coefficient of the SDS micelles as the bile salts are included.

At higher mole fractions of bile salts there exists the possibility of formation of bile salts aggregates. SDS molecules can interact with the hydrophobic moieties of the bile salts in the interior of the micelles. In other words, SDS molecules modify the steroidal backbone or aggregate within the bile salts micelles, leaving the outside HBA hydroxyl functional groups available for hydrogen bonding interactions. This explains the similar LSER *a*-coefficients for the individual bile salts and the mixed micelles. In addition, the nature of the SDS aggregates is different in the mixed systems as compared to the pure SDS micelles, especially in terms of the localized water molecules. Carey and Small [26] illustrated a similar structure for a mixture of sodium oleate and sodium deoxycholate with the alkyl chain surfactant modifying the interior hydrophobic moiety of the bile salt micelles.

In addition to their effects on retention and selectivity, one should also note that the size of the elution window for the mixed micellar pseudo-stationary phases can be very different from the individual constituents. As shown in Fig. 8, the size of the elution windows of mixed SDC–SDS micellar systems at various mole fractions and total surfactant concentrations are larger than either of the two individual systems at the same conditions. As a result, resolution in these mixed systems is improved as compared to the individual systems [15]. Apparently, the charge-to-mass ratio of the mixed SDS–bile salts is greater than either SDS or bile salts micelles. This leads to larger micellar mobility and a wider elution window.
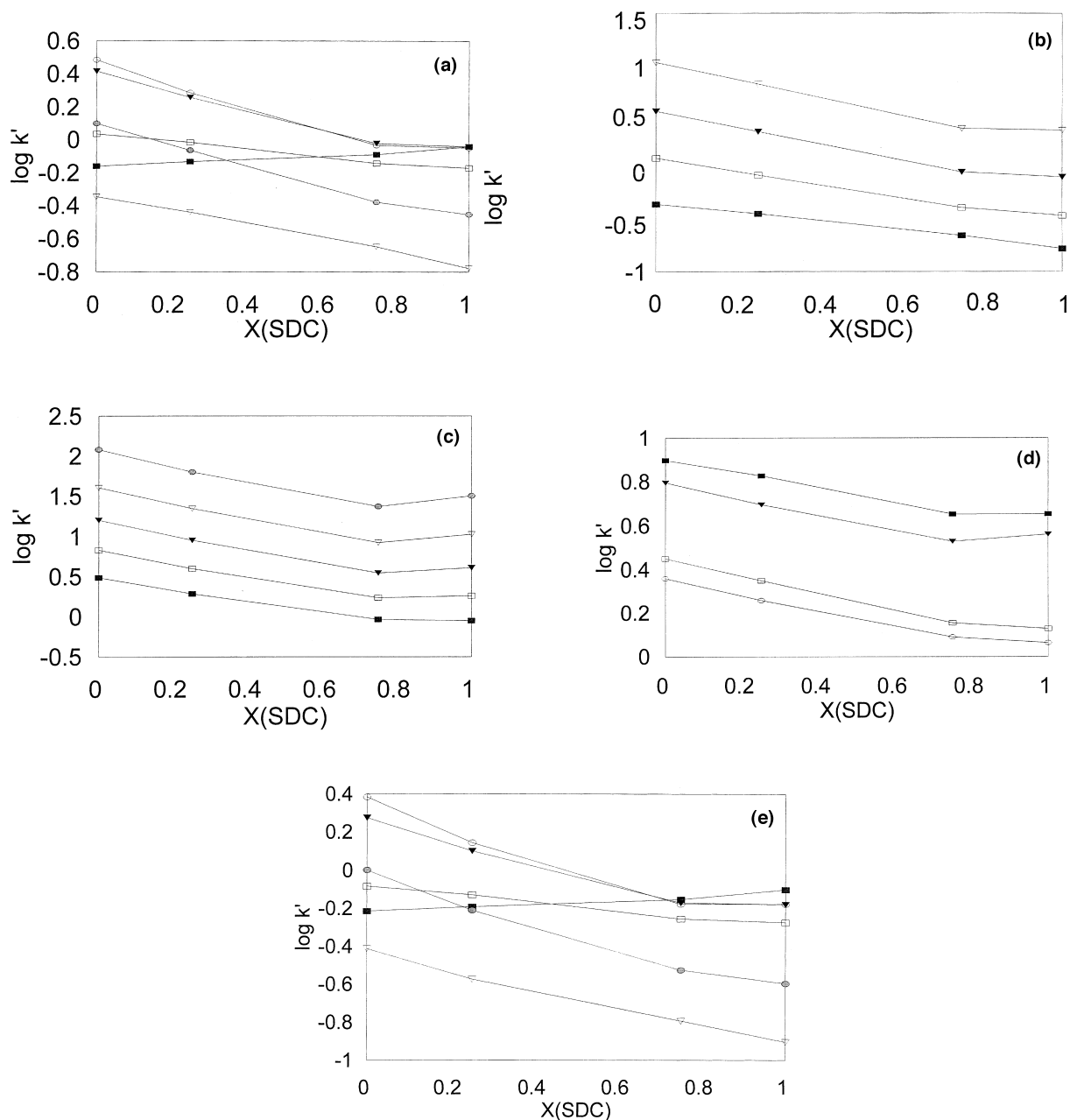
Fig. 6. Influence of composition of SDS/SDC mixed micelles [mole fraction of SDC, X(SDC)] on MEKC retention factor (log k′) for: (a) solutes with a hydrogen bond functional group: acetophenone (empty circle), nitrobenzene (filled triangle), nitrobutane (dashed circle), phenol (empty square), resorcinol (filled square), and nitropropane (empty triangle). (b) Homologous series of nitroalkanes: nitropropane (filled square), nitrobutane (empty square), nitropentane (filled triangle), and nitrohexane (empty triangle). (c) Homologous series of alkyl aryl ketones: acetophenone (filled square), propiophenone (empty square), butyrophenone (filled triangle), valerophenone (empty triangle), and hexaphenone (filled circle). (d) Nonhydrogen bond solutes: benzene (empty circle), fluorobenzene (empty square), toluene (filled triangle), and chlorobenzene (filled square). Total surfactant concentration was 100 mM, pH=9.0. (e) Same as (a), except total concentration=75 mM (b).
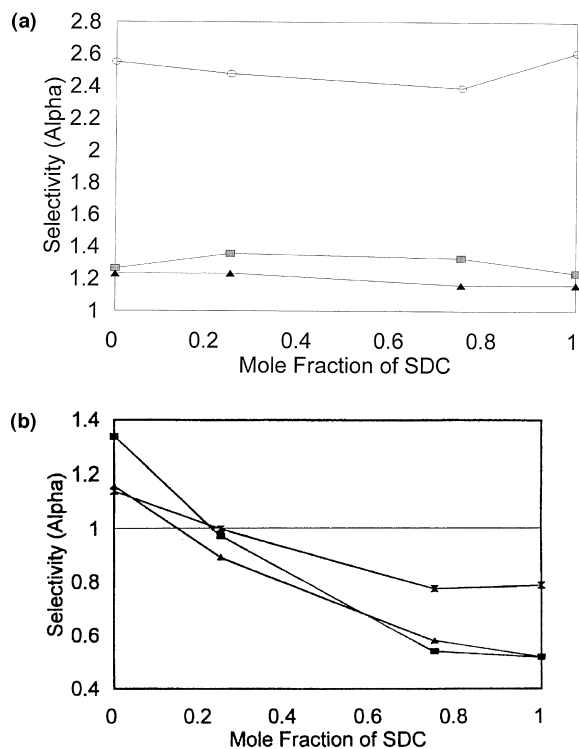
(a)

(b)

Fig. 7. Influence of mole fraction of SDC [X(SDC)] on selectivity, α, in mixed micellar EKC with SDS–SDC mixed micelles between: (a) NHB solute pairs. Compound pair identifications: nitrobutane–nitropropane (empty circle), chlorobenzene–toluene (square), benzene–fluorobenzene (triangle). (b) NHB, HBD and HBA solute pairs. Compound pair identifications: nitrohexane–chlorobenzene (square), benzene–nitrobenzene (hourglass), phenol–nitrobutane (triangle).

Fig. 8. Comparison between the size of the elution windows (t_{mc}/t_{eo}) of individual and mixed surfactant systems of SDS and SDC versus total surfactant concentration at various mole fractions of SDC. Corresponding values of the mole fraction of SDC are: 0 (filled square), 0.25 (plus), 0.75 (asterisk), and 1 (open square).

Acknowledgements

4. Conclusions

The LSER modeling of MEKC retention has been very useful for characterization of mixed bile salts and SDS/bile salt MEKC systems. Type and composition of pseudostationary phase play a vital role in elution patterns of solutes in MEKC. Due to the existence of a variety of combinations of pseudostationary phases, selection of the appropriate micellar composition for a given separation is difficult. Characterization of individual as well as mixed surfactant systems is necessary for the timely and accurate selection of pseudostationary phases in MEKC.
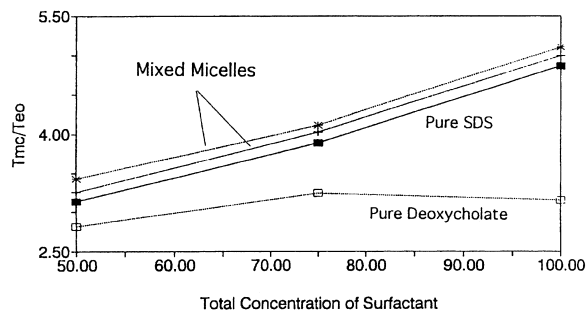
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