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Mixed micelles of short chain alkyl surfactants and bile salts in electrokinetic chromatography: Enhanced separation of corticosteroids

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Abstract

The separation of a complex mixture of 17 corticosteroids was investigated by mixed micellar electrokinetic chromatography (MMEKC) employing various bile salts and/or alkylsulfonates. In this study, influence of individual surfactants and mixed micelles of hydrocarbon–bile salt surfactants on retention behavior, selectivity and the size of the elution window is investigated. Retention behavior of corticosteroids in SDS and bile salt micelles is examined using linear solvation energy relationships (LSER). In addition, the effects of type of bile salt surfactant on elution patterns were investigated. It was found that separation patterns are mostly influenced by the number of hydroxyl functional groups on the steroidal backbone of the bile salts, while the type of ionic head group has little, if any, effect on the steroids separation. Comparisons between mixed micellar techniques and the inclusion of conventional modifiers to various single and binary surfactant systems were made. The addition of modifiers such as acetonitrile, urea and β-cyclodextrin to SDS surfactant systems, as well as mixed bile salt systems of sodium taurocholate and sodium glycodeoxycholate, did not improve the separation of the steroids. On the other hand, the addition of the short-chain alkylsulfonate sodium butanesulfonate to the mixture of taurocholate and glycodeoxycholate greatly improved the separation of the 17 corticosteroids and provided a baseline separation of all solutes. The effects of carbon chain length and concentration of alkylsulfonate on capacity factor, selectivity, efficiency and the size of the elution window were investigated.

Keywords: Micelles, mixed; Linear solvation energy relationships; Corticosteroids; Surfactants; Bile salts; Alkylsulfonates; Organic modifiers; Cyclodextrins

1. Introduction

Over the past decade, the popularity of micellar electrokinetic chromatography (MEKC) has increased dramatically primarily because of its ability to separate uncharged species on the basis of their differential partitioning into a micellar pseudostationary phase [1,2]. The type of surfactant has a great influence on separations since the migration of uncharged species in MEKC is due to the extent of their interactions with an ionic micellar phase. The most commonly used surfactant in MEKC is sodium dodecyl sulfate (SDS). The incorporation of modifiers such as organic solvents [3–5], cyclodextrins [6,7] and urea [8] into SDS-MEKC systems is an effective method for improving separations of hydrophobic compounds such as polycyclic aromatic hydrocarbons (PAHs) and PTH-amino acids. Al-

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though SDS–modifier systems are useful in many cases, they are not applicable to all situations. Bile salt surfactants offer a viable alternative to conventional alkyl surfactants such as SDS due to their specific structural properties such as type and number of functional groups [9–11]. Individual bile salt surfactants are useful for the separation of dansyl-amino acids [12], benzothiazepin analogs [13], PAHs [14], as well as active ingredients in cold remedies [15]. Bile salts have also been shown to be useful for the separation of a mixture of eight corticosteroids [13]. An alternative to individual surfactant systems or the use of modifiers for the separation of complex mixtures of structurally similar compounds are mixed micellar systems. Mixed micellar electrokinetic chromatography (MMEKC) involves the use of mixtures of surfactants with various structural properties in the running buffer in MEKC. By tailoring micellar environments, solute–micelle interactions can be manipulated, leading to a greater control over migration behavior in MEKC. MMEKC has been shown to be useful in various reports [16–20] for the separation of groups of compounds such as homologous series, amino acids and peptides. Previously, the separation of a group of 17 corticosteroids using various individual and mixed micellar systems of bile salts and/or SDS was investigated [21]. Mixed bile salt systems of sodium taurocholate, sodium glycodeoxycholate and SDS were used to partially separate all 17 corticosteroids. Despite offering extended elution windows (t_{mc}/t_{eo}) and improved separations with a group of structurally similar solutes, mixed bile salt–SDS micelles did not provide complete separation of the complex mixture of 17 corticosteroids [21]. In this work, linear solvation energy relationships (LSERs) are used to examine characteristics of SDS and bile salts for the separation of corticosteroids. Addition of modifiers to SDS, as well as bile salt surfactant systems were examined for possible improvement of the separation of corticosteroids. It is shown in this work that the inclusion of short chain alkylsulfonates into mixed bile salt buffers leads to the baseline separation of the mixture of all 17 solutes. By examining characteristics such as capacity factor, selectivity, efficiency and the size of the elution window of specific micellar systems, changes in resolution resulting from the addition of alkylsulfonates to mixtures of bile salt surfactants can be better understood. Also, the effect of structural differences in bile salts such as the number of hydroxyl groups and the type of ionic head group on separations of corticosteroids were examined.

2. Experimental

2.1. Apparatus

A laboratory-made CE system was used. It consisted of 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, Scientific Systems (SSI), State College, PA, USA) operating at 254 nm, and 53 μm I.D.×357 μm O.D. fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA). The total length of the capillary was 62 cm and the length of the capillary to the detector was 50 cm. An applied voltage of 15 kV was used unless otherwise stated and the capillary was left at ambient temperature. Electropherograms were collected with an electronic integrator (Hewlett-Packard, Avondale, PA, USA).

2.2. Reagents

Sodium alkylsulfonates were purchased from Lancaster Synthesis (Windham, NH, USA) and were used as received. Bile salts were obtained from Aldrich (Milwaukee, WI, USA) and Sigma (St. Louis, MO, USA). Seventeen corticosteroids were obtained from Sigma and Steraloids (Wilton, NH, USA). β-Cyclodextrin was purchased from Sigma. Urea was purchased from Aldrich. Acetonitrile was obtained from Fisher (Raleigh, NC, USA). All buffer solutions were prepared from analytical grade reagents and water was purified with a Milli-Q system and filtered through a 0.45-μm filter. HPLC-grade methanol (Fisher) was used to mark the electroosmotic flow (t_{eo}) and 1-nitropyrene (Aldrich) was used to track the micelle migration (t_{mc}).

2.3. Procedure

All experiments were performed at pH 9.0 using a 50 mM ionic strength phosphate–borate buffer. The composition of the running buffer included sodium

phosphate monobasic and boric acid, and was adjusted to pH 9.0 with sodium hydroxide. The relative amounts of each of the buffer components was determined by using the computer program BUFCALC (a program developed in this laboratory in Turbo Vision). Stock solutions of the test solutes were dissolved in methanol at a concentration of 5 mg/ml. Running concentrations of the test solutes were 250 μg/ml. The capillary was rinsed with buffer solution between each run for approximately 1 min and was rinsed with methanol, sodium hydroxide (1 *M*), and water between buffers. Capacity factors (k') were calculated using Eq. 1:

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

3. Results and discussion

According to Eq. 2, resolution in MEKC can be influenced by efficiency (N), capacity factor (k'), selectivity (α) and the size of the elution window (t_{mc}/t_{eo}).

$$R_s = \left(\frac{N^{1/2}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 + k'_2}\right)\left(\frac{1 - \left(\frac{t_{eo}}{t_{mc}}\right)}{1 + \left(\frac{t_{eo}}{t_{mc}}\right)k'_1}\right) \quad (2)$$

By altering parameters such as type of surfactant, total concentration of surfactant and the composition of the micellar system, factors such as selectivity, capacity factor and the size of the elution window may be adjusted, which in turn affect resolution.

3.1. SDS vs. bile salts

Retention behavior of a group of 17 corticosteroids has been shown to be very different in SDS and bile salt surfactant systems [21]. The hydrophobicities of the corticosteroids chosen as test solutes for this work range from $\log P_{ow} = 1.03$ to $\log P_{ow} = 3.87$. Of these test solutes, 16 are moderately hydrophobic with $\log P_{ow}$ values between 1 and 3. Only progesterone is very hydrophobic, having a $\log P_{ow}$ value above 3. Despite the fact that most of these compounds are only moderately hydrophobic, it has been shown that they elute at or near t_{mc} in SDS systems, while eluting earlier in bile salt systems [21]. Recently, the influence of different types of surfactants on migration behavior was studied using LSERs [22,23]. In general, an LSER model can be set up using Eq. 3 in which a solvent-related property of a solute (SP) can be described by four coefficients associated with the solvent system (m, s, b, a) and four solute descriptors (V_1, π^*, β, α).

$$SP = SP_o + mV_1/100 + s\pi^* + b\beta + a\alpha \quad (3)$$

In our case, the logarithm of the capacity factor (k') is used as SP and is correlated with the solute's molar volume (i.e., size) and three solvatochromic parameters (π^*, β, α) using multiple linear regression. The π^* descriptor represents the dipolarity/polarizability of solutes, β is a measure of solute hydrogen bond acceptor strength (basicity) and α is the solute hydrogen bond donor strength (acidity). The coefficients obtained from linear regression (m, s, b, a) describe properties of the surfactant system used. Recently, SDS and sodium cholate (SC) systems were investigated [22,23] using LSER analysis. The following equations were obtained to describe these systems:

A 40 m*M* SC-MEKC system:

$$\log k' = -1.49 + 3.95V_1/100 - 0.26\pi^* - 1.80\beta - 0.18\alpha$$

$n = 60$, $r = 0.9553$, S.E. $= 0.156$

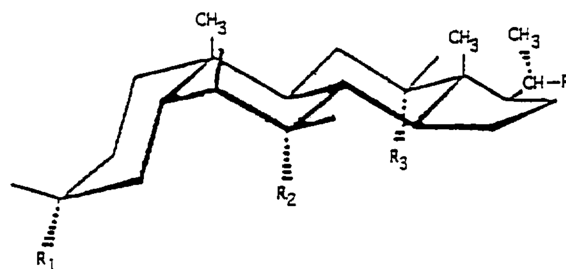
a 60 m*M* SC-MEKC system:

$$\log k' = -1.62 + 3.89V_1/100 - 0.27\pi^* - 2.88\beta + 0.23\alpha$$

$n = 60$, $r = 0.9684$, S.E. $= 0.144$

By examining these LSER results, the retention behavior of corticosteroids in these two systems can be better understood. From the above equations, it can be seen that SC and SDS systems have extremely similar m and s values. Terms that exhibit significant differences from one another are b and a, the hydrogen bonding interaction terms, and SP_o, which contains information about phase ratio. From the relationships shown above, it was concluded that SC is a stronger hydrogen bond acceptor (HBA) surfactant system than SDS (SC has a larger positive

a coefficient), while SDS is a stronger hydrogen bond donor (HBD) surfactant system than SC (SDS has a less negative *b* coefficient) [22,23]. The long retention times the steroids exhibit with SDS systems as compared to SC systems can be attributed to differences in hydrogen bonding interactions between the two systems and the steroids, as well as differences in phase ratio between the two systems. Corticosteroids are bulky molecules (large V) and contain hydrogen bond acceptor groups such as carbonyl or hydroxyl. As a result, the HBA corticosteroids have stronger hydrogen bond interactions with HBD–SDS surfactant systems and weaker interactions with HBA–SC systems, which partly accounts for the reduced k' values of the steroids in SC surfactant systems as compared to SDS surfactant systems. Also, SDS systems have larger phase ratios than SC systems at the same total surfactant concentration. As a result of a combination of the two above mentioned characteristics of these surfactant systems, a better separation of the 17 test solutes can be achieved by using a 100 m*M* bile salt surfactant system as compared to a 100 m*M* SDS surfactant system [21].



General Bile Salt Structure

Bile Salt (Sodium)	R1	R2	R3	R
Taurocholate	-OH	-OH	-OH	$-CH_2CH_2CONHCH_2CH_2SO_3^-$
Glycodeoxycholate	-OH	-H	-OH	$-CH_2CH_2CONHCH_2COO^-$
Taurodeoxycholate	-OH	-H	-OH	$-CH_2CH_2CONHCH_2CH_2SO_3^-$
Cholate	-OH	-OH	-OH	$-CH_2CH_2COO^-$
Deoxycholate	-OH	-H	-OH	$-CH_2CH_2COO^-$

Fig. 1. Structures of bile salt surfactants.

3.2. *Influence of bile salt structure*

Different retention behavior can be observed for various bile salt micelles in MEKC. The structures of bile salt surfactants employed for this study are shown in Fig. 1. Bile salts are composed of a hydrophobic steroid backbone possessing various hydrophilic functional groups. As shown in Fig. 1, bile salts used in this study differ in either their ionic head group, the number of hydroxyl groups they have, or both. Taurocholate is a tri-hydroxy bile salt with a sulfonate terminating head group, while glycodeoxycholate is a di-hydroxy bile salt with a carboxyl terminating head group. Therefore, these two bile salts have different numbers of hydroxyl groups and different ionic head groups. As shown previously [21], 100 m*M* taurocholate provides a very different separation for the group of 17 steroids than 100 m*M* glycodeoxycholate. In order to determine which of the structural differences of the bile salts has the most effect on the elution pattern of the mixture, the bile salt taurodeoxycholate was compared with taurocholate, as well as glycodeoxycholate in single and binary bile salt solutions. Taurodeoxycholate has the same number of hydroxyl groups as glycodeoxycholate and the same ionic head group as taurocholate. The elution pattern of the steroids using 100 m*M* taurodeoxycholate is identical to 100 m*M* glycodeoxycholate, but very different from 100 m*M* taurocholate [21]. Similarly, a binary mixture of glycodeoxycholate and taurodeoxycholate at a total concentration of 100 m*M* results in the same separation pattern of the 17 steroids as provided by the two individual surfactant systems. As shown in Table 1, the two individual surfactants, as well as their mixed micelles, provide similar k' and α values for the steroids. As a result, it can be concluded that differences of the terminals of the ionic head groups of bile salts has very little, if any, effect on different separation patterns. On the other hand, differences in hydroxyl groups of bile salts lead to very different elution patterns for the steroids and influence the size of the elution window, as well as solutes' retention and selectivity greatly.

Table 1
Values of migrations factors (k') and selectivity (α) for eleven corticosteroids in three surfactant systems

Steroid	System A		System B		System C	
	k'	α	k'	α	k'	α
Progesterone	35.15		34.10		38.99	
		2.74		2.60		2.55
Deoxycorticosterone	12.81		13.12		15.30	
		1.87		1.85		1.95
6α-Methylprednisolone	6.85		7.10		7.84	
		1.24		1.23		1.22
Corticosterone	5.54		5.77		6.40	
		1.41		1.22		1.20
Fludrocortisone acetate	3.92		4.72		5.33	
		1.07		1.23		1.29
Prednisolone	3.68		3.81		4.13	
		1.00		1.00		1.00
Hydrocortisone	3.68		3.81		4.13	
		1.13		1.13		1.13
Fludrocortisone	3.25		3.38		3.65	
		1.16		1.17		1.18
Cortisone	2.79		2.89		3.10	
		1.09		1.09		1.09
Prednisone	2.56		2.66		2.85	
		1.76		1.90		2.28
Triamcinolone	1.45		1.40		1.24	

System A, 100 mM glycodeoxycholate; System B, 50 mM glycodeoxycholate–50 mM taurodeoxycholate; System C, 100 mM taurodeoxycholate.

In order to further verify the effect of hydroxyl group number on the separation of corticosteroids, two additional bile salts, sodium cholate (SC) and sodium deoxycholate, were examined. A total surfactant concentration of 100 mM with different mole fractions of each bile salt were used. Cholate and deoxycholate have identical carboxy-terminating ionic head groups and differ only by their number of hydroxyl groups. As shown in Fig. 2, not only do capacity factors for the corticosteroids increase as the mole fraction of deoxycholate in the mixed micellar systems is increased, but the relative difference between k' values for certain pairs of compounds increases. This indicates that selectivity is altered by changing the composition of the mixed cholate–deoxycholate micellar system. As shown in Fig. 3, selectivity between some pairs of steroids is altered to the point of peak elution reversal by changing the composition of the micellar system. This further supports that changes in elution pattern for a group of corticosteroids using mixed bile salt systems are

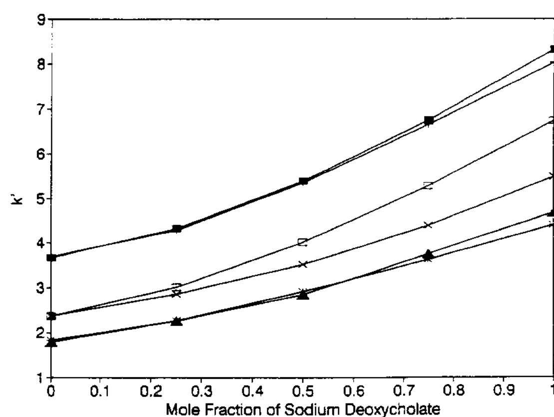


Fig. 2. Effect of mole fraction of sodium deoxycholate in mixed micellar systems of sodium cholate and deoxycholate on capacity factor (k'). Total surfactant concentration is 100 mM. Peak identifications: 6α-methylprednisolone (■); fluocinolone acetonide (+); prednisolone (*); corticosterone (□); prednisolone acetate (×); hydrocortisone (▲). Conditions: capillary, 50 cm effective length×53 μm I.D.; temperature, ambient; detection wavelength, 254 nm; applied voltage, 15 kV; buffer, 0.05 M phosphate–borate buffer at pH 9.0.

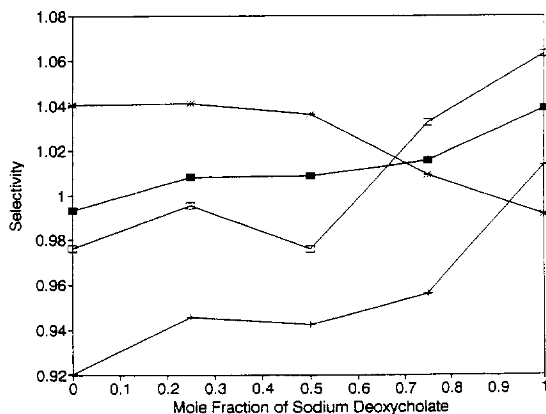


Fig. 3. Effect of mole fraction of sodium deoxycholate in mixed micellar systems of sodium cholate and deoxycholate on selectivity (α). Total surfactant concentration is 100 m*M*. Peak pair identifications, 6α-methylprednisolone and fluocinolone acetonide (■); prednisolone acetate and hydrocortisone-21-acetate (*); triamcinolone acetonide and fluocinolone acetonide (+); hydrocortisone and prednisolone (□). Other conditions as described in the legend to Fig. 2.

due to differences in the hydroxyl functional groups on bile salt surfactants.

3.3. Effect of modifiers

Traditionally, a method of improving resolution in MEKC systems is the addition of modifiers to the micellar running buffer. In an effort to improve the separation of the corticosteroids, three modifiers, β-cyclodextrin, urea and acetonitrile, were studied in conjunction with various surfactant systems. When these modifiers were examined with 75 m*M* SDS, corticosteroid separations were slightly improved over separations using only SDS, but were inferior to those reported previously using mixed micellar systems [21]. Fig. 4a shows the separation of the 17 steroids using an SDS systems including 5% acetonitrile. A mixture of sodium taurocholate and sodium glycodeoxycholate offers a partial separation of all 17 steroids, but does not separate all 17 steroids with baseline resolution [21]. Addition of modifiers (β-cyclodextrin, urea and acetonitrile) to the mixed bile salt micelles of taurocholate and glycodeoxycholate offers no improvement in steroid separation, as illustrated in Fig. 4b.

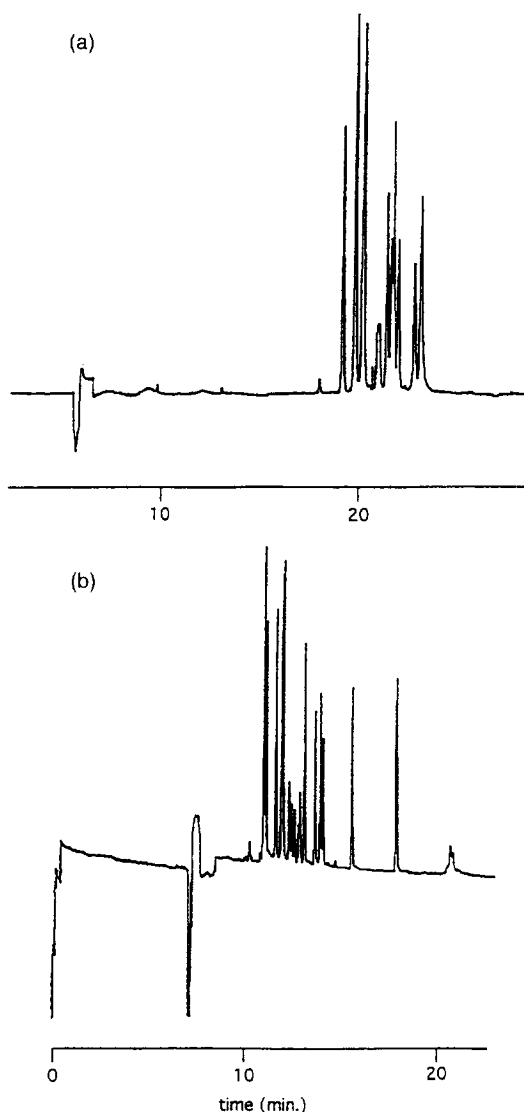


Fig. 4. Separation of 17 corticosteroids. (a) 75 m*M* SDS and 5% acetonitrile; (b) 33 m*M* taurocholate, 33 m*M* glycodeoxycholate, 5% acetonitrile. Other conditions as described in the legend to Fig. 2.

3.4. Mixed micelles of bile salts and alkyl surfactants

Previously it was shown that a binary mixture of taurocholate and glycodeoxycholate resulted in a partial separation of a group of 17 corticosteroids [21]. It was also noted that the addition of SDS to the binary mixture of taurocholate and glycodeoxy-

cholate resulted in an increase in the size of the elution window (t_{mc}/t_{eo}). Unfortunately, the gain in resolution due to a larger elution window was offset as a result of a loss in selectivity between several peaks and/or larger k' values. In order to maintain the large elution window without a loss in selectivity or an increase in k', the use of shorter chain anionic alkyl surfactants was investigated. In this work, it is shown that the addition of short-chain alkylsulfonate surfactants to the above-mentioned mixture of taurocholate and glycodeoxycholate improves the separation of the 17 steroids. Sodium dodecyl sulfate (SDS) and sodium dodecylsulfonate differ only in their ionic head groups. Because of this similarity, alkylsulfates and alkylsulfonates with the same chain length have very similar critical micelle concentrations (CMC). The CMC values of SDS and dodecylsulfonate are 8.1 and 9.8 m*M*, respectively [24]. Short-chain alkylsulfonates were used as opposed to short-chain alkylsulfates because of the availability of the shorter chain homologues and cost considerations. Five sodium salts of alkylsulfonates (butane-, hexane-, octane-, decane- and dodecanesulfonates) were examined in conjunction with the binary bile salt system of 33 m*M* taurocholate and 33 m*M* glycodeoxycholate. As shown in Fig. 5, as the length of the carbon chain of the alkylsulfonate increases, the size of the elution window (t_{mc}/t_{eo}) increases (systems 1–5). This is a result of both an increase in electroosmotic flow (smaller t_{eo}) and an increase in the retention time of the micelle (larger t_{mc}). This increase in the size of the elution window may possibly be attributed to an increase in the charge-to-mass ratio of the mixed micelles. Bile salt monomers have bulky steroid backbones with only one charged head group. The aggregation numbers of the bile salt micelles are between 3 and 14 [24], which means that the charge to mass ratio is small for the bile salt micelles. The alkylsulfonates, on the other hand, are small alkyl chains with a sulfonate head group. Typical alkylsulfonate or alkylsulfate micelles such as SDS have aggregation numbers close to 50 or 60 [24]. As the tail of the alkylsulfonate gets longer or becomes more hydrophobic, more monomers will be attracted to the hydrophobic portions of the bile salt micelles, thus increasing the charge to mass ratio of the mixed micelles. This increase in negative charge will

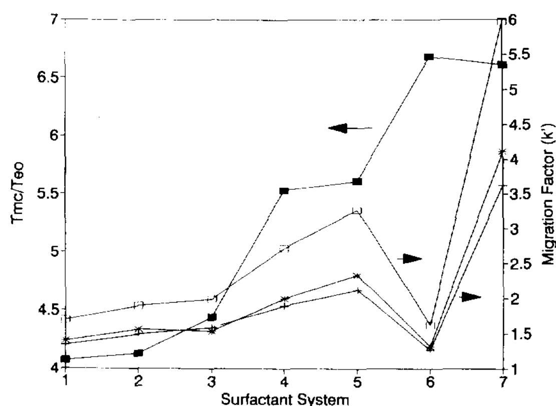


Fig. 5. Comparison between the size of elution windows (t_{mc}/t_{eo}; ■) and k' of various surfactant systems. Steroid identifications: Prednisone (+); cortisone (*); fludrocortisone (□). Surfactant system identifications: 33 m*M* taurocholate, 33 m*M* glycodeoxycholate, and (1) 33 m*M* butanesulfonate, (2) 33 m*M* hexanesulfonate, (3) 33 m*M* octanesulfonate, (4) 33 m*M* decanesulfonate, (5) 33 m*M* dodecanesulfonate, (6) 70 m*M* butanesulfonate, (7) 70 m*M* dodecanesulfonate. Other conditions as described in the legend to Fig. 2.

increase the mobility of the mixed micelle in the opposite direction of the electroosmotic flow and causes the t_{mc} marker to elute later. The size of the elution window would further increase with the concentration of alkyl surfactant. The largest value for the size of the elution window occurs with the system of 33 m*M* taurocholate, 33 m*M* glycodeoxycholate and 70 m*M* butanesulfonate (system 6 in Fig. 5). This may be due to a further increase in the charge-to-mass ratio of the micelle because of the drastic increase in the concentration of short-chain alkylsulfonate from 33 to 70 m*M*. The elution window obtained when using 70 m*M* dodecanesulfonate instead of butanesulfonate with the mixture of two bile salts is approximately the same. This may be due to the saturation of the bile salt micelle with alkylsulfonate monomers or due to micelle formation of the longer chain surfactant. When 90 m*M* butanesulfonate was used in place of 70 m*M* butanesulfonate with the mixed bile salt system, no further increase in the size of the elution window occurred, further supporting the above-mentioned hypotheses. According to Fig. 5, k' increases as the number of carbons in the chain of the alkylsulfonate increases. It should be noted that the values for capacity factor in the 70 m*M* butanesulfonate–mixed

bile salt system are approximately the same as the values for the 33 mM butanesulfonate–mixed bile salt system. According to Terabe [2], because of the limited elution window in MEKC, there is an optimum range of capacity factor values at a given elution window size which will result in the highest resolution. For uncharged solutes, it has been determined that the optimum range for k' values is between 2 and 5 [25]. Fig. 5 shows that as the alkylsulfonate chain length increases, some k' values clearly move away from the optimum range. However, the 70 mM butanesulfonate–mixed bile salt system offers an increase in the size of the elution window while maintaining k' values in or near the optimum range. It should be noted that values of capacity factors are similar for butanesulfonate, hexanesulfonate and octanesulfonate systems, but change dramatically for decanesulfonate and dodecanesulfonate systems. A possible explanation for this change in k' in systems with higher chain length alkylsulfonates is the micelle formation of the higher chain length additives. Butane and hexanesulfonate are short-chain alkylsulfonates, and as a result do not form micelles at concentrations used in this work. It is clear that only alkylsulfonates with chain lengths longer than 8 form micelles at concentrations used in this work, since the highest concentration of any additive used was 90 mM. The aggregation of long chain alkyl surfactant would obviously influence retention of solutes. The shorter chain alkylsulfonates can only modify the bile salt micelles. As shown in Fig. 6, the efficiency of an early eluting compound (fludrocortisone), middle eluting compound (triamcinolone acetonide) and late eluting compound (progesterone) decreases as the length of the alkylsulfonate chain increases in almost all cases. Reduced plate counts were observed for the 70 mM butanesulfonate–mixed bile salt system, but still were calculated to be at least 70 thousand theoretical plates per meter. This decrease in efficiency is probably due to Joule heating at high concentration of butanesulfonate. Despite this decrease in efficiency, the 70 mM butanesulfonate–mixed bile salt system still resulted in a baseline separation of the mixture of 17 steroids as shown in Fig. 7. This can be attributed to the increase in elution window, optimum k' values and optimum selectivity that this system offers, which compensates for the loss in efficiency.

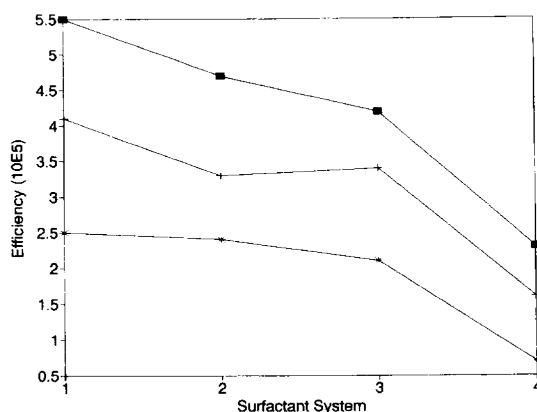


Fig. 6. Average number of theoretical plates per meter ($\times 10^5$) calculated for three steroids in various surfactant systems. Steroid identifications: fludrocortisone (■); triamcinolone acetonide (+); progesterone (*). Surfactant system identifications: 33 mM taurocholate, 33 mM glycodeoxycholate, and (1) 33 mM butanesulfonate, (2) 33 mM octanesulfonate, (3) 33 mM dodecanesulfonate, (4) 70 mM butanesulfonate. Other conditions as described in the legend to Fig. 2.

4. Conclusions

In this work it was shown that SDS and bile salt surfactant systems provide very different MEKC elution patterns for the mixture of 17 structurally similar corticosteroids. The variations in selectivity can be attributed to differences in hydrogen bonding characteristics of these systems. It was also shown that bile salts with different structural characteristics such as their number of hydroxyl groups provide different separations of the corticosteroids. It was demonstrated that MMEKC is a viable alternative to conventional anionic alkyl surfactant–modifier buffers for the separation of corticosteroids. The addition of short-chain ionic alkyl surfactant to bile salt micelles seems to be an effective method for enhancing the elution window of bile salt micelles without adverse effects on retention and selectivity of the system.

Acknowledgments

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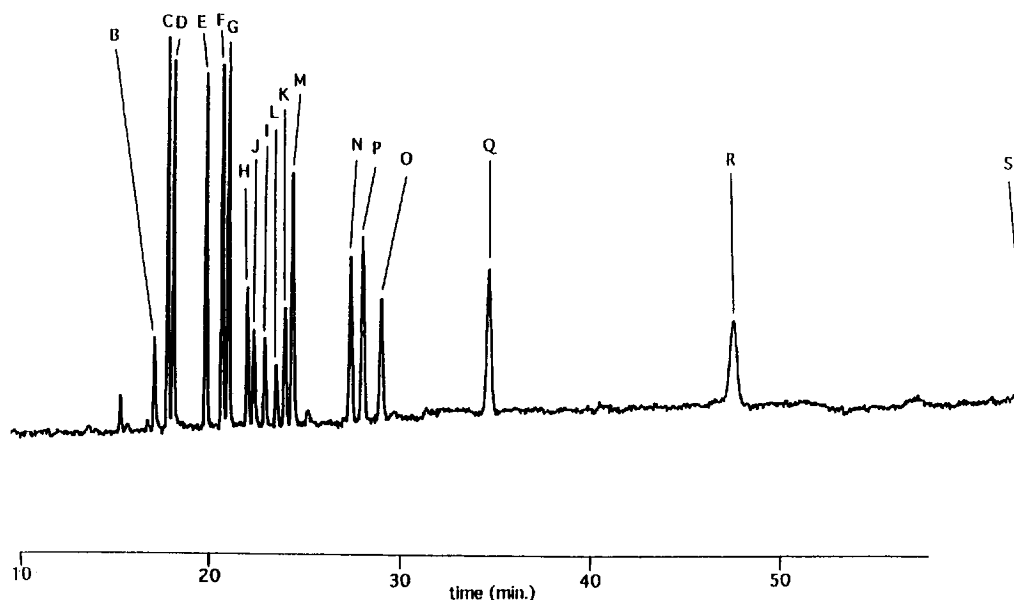

Fig. 7. Separation of 17 corticosteroids. Buffer, 33 mM taurocholate–33 mM glycodeoxycholate–70 mM butanesulfonate. Peak identifications: (A) electroosmotic flow marker (t_{eo}); (B) triamcinolone; (C) prednisone; (D) cortisone, (E) fludrocortisone; (F) hydrocortisone; (G) prednisolone; (H) prednisone acetate; (I) fludrocortisone acetate; (J) cortisone acetate; (K) prednisolone acetate; (L) hydrocortisone-21-acetate; (M) corticosterone; (N) triamcinolone acetonide; (O) fluocinolone acetonide; (P) 6α-methyl prednisolone; (Q) deoxycorticosterone; (R) progesterone; (S) micellar marker (t_{mc}). Other conditions as described in the legend to Fig. 2.

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