Journal of Chromatography A, 1217 (2010) 375–385

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Binary mixed micelles of chiral sodium undecenyl leucinate and achiral sodium undecenyl sulfate: I. Characterization and application as pseudostationary phases in micellar electrokinetic chromatography

David M. Ahlstrom [a], Yatzka M. Hoyos [a], Hakan Arslan [a,b], Cevdet Akbay [a,*]

[a] Department of Natural Sciences, Fayetteville State University, 1200 Murchison Road, Fayetteville, NC 28301, USA
[b] Department of Chemistry, Faculty of Pharmacy, Mersin University, Mersin, TR 33169, Turkey

ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form 6 November 2009
Accepted 10 November 2009
Available online 13 November 2009

Keywords:
C_{20}
CMC
γ_{CMC}
Hydrogen bond acceptor
Hydrogen bond donator
Linear solvation energy relationships
Micellar electrokinetic chromatography
Mixed surfactant systems
Non-hydrogen bond donator
Partial specific volume
Pseudostationary phase
Sodium 10-undecenyl leucinate
Sodium 10-undecenyl sulfate
Surfactant

ABSTRACT

Sodium 10-undecenyl sulfate (SUS), sodium 10-undecenyl leucinate (SUL) and their five different mixed micelles at varied percent mole ratios were prepared. The critical micelle concentration (CMC), C_{20}, γ_{CMC}, partial specific volume, methylene group selectivity, mobilities and elution window were determined using a variety of analytical techniques. These surfactant systems were then evaluated as novel pseudostationary phases in micellar electrokinetic chromatography (MEKC). As a commonly used pseudostationary phase in MEKC, sodium dodecyl sulfate (SDS) was also evaluated. The CMC values of SUS and SUL were found to be 26 and 16 mM, respectively, whereas the CMC of mixed surfactants was found to be very similar to that of SUL. The C_{20} values decreased dramatically as the concentration of SUL is increased in the mixed micelle. An increase in SUL content gradually increased the methylene group selectivity making the binary mixed surfactants more hydrophobic. Linear solvation energy relationships (LSERs) and free energy of transfer studies were also applied to predict the selectivity differences between the surfactant systems. The cohesiveness and the hydrogen bond acidic character of the surfactant systems were found to have the most significant influence on selectivity and MEKC retention. The SUS and SDS showed the strongest while SUL showed the weakest hydrogen bond donating capacity. The basicity, interaction with *n* and π-electrons of the solute and dipolarity/polarizability were the least significant factors in LSER model for the surfactant systems studied. Free energies of transfer of selected functional groups in each surfactant systems were also calculated and found to be in good agreement with the LSER data.

Published by Elsevier B.V.

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a separation technique that combines the powerful features of liquid chromatography and capillary electrophoresis [1,2]. In MEKC, neutral and charged solutes can be separated simultaneously based on their differential partitioning between the mobile phase and the pseudostationary phase. The mobile phase is an aqueous buffer, whose properties can be manipulated by addition of modifiers such as organic solvents, cyclodextrins, urea, chiral additives etc. The pseudostationary phase is usually a charged surfactant that is added to the mobile phase at concentration above its critical micelle concentration (CMC). One of the major advantages of MEKC over other common separation techniques is the viability of optimizing the selectivity by simply rinsing the separation capillary with a new separation buffer solution containing a desired surfactant. Sodium dodecyl sulfate (SDS), an anionic surfactant, has been widely used as a pseudostationary phase in many MEKC applications.

One of the successful approaches to modify the selectivity in MEKC has been the selection of a surfactant of different nature. To date, a number of new pseudostationary phases with diverse selectivities have been introduced as alternative to SDS [3,4]. Altering the counter ion of the surfactant [5–7], addition of organic solvents, urea and cyclodextrins [8–11] are found to be useful for selectivity modification of a given pseudostationary phase. Another effective approach to improve the selectivity in MEKC is the application of mixed micelles of two or more different surfactants [3,12]. The mixed surfactant systems can be especially advantageous when their constituents have diverse properties.

Selection of proper surfactant for separation of special chemicals with varied physicochemical properties requires an understanding of the nature of solute-micelle interaction. Linear solvation energy relationships (LSERs) model has been introduced as a powerful tool for the characterization of retention and selectivity of

* Corresponding author. Tel.: +1 910 672 1943; fax: +1 910 672 1159.
E-mail address: cakbay@uncfsu.edu (C. Akbay).

0021-9673/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.chroma.2009.11.023

pseudostationary phases in MEKC [13–18]. Initially developed by Kamlet et al. [19,20], this model provides information about the physicochemical properties of the separation systems as well as the magnitude of the different interactions between the pseudostationary phases and the solutes. More recently, Platts et al. [21] showed improved accuracy of some of the solute descriptors with new symbols and modified the LSER model which can be written as

$$\log k = c + vV + eE + sS + aA + bB \quad (1)$$

where V, E, S, A, and B are known as Abraham solute descriptors and are correlated to the logarithmic retention factor ($\log k$). V and E are measures of a solute's McGowan's characteristic volume and the excess molar refraction, respectively. The solute dipolarity/polarizability is represented by the S term. The A and B terms represent the hydrogen bond acidity (donating ability) and the hydrogen bond basicity (accepting ability) of the solute, respectively. The system coefficients c, v, e, s, a, and b refer to differences in the aqueous buffer and the pseudostationary phases, between which the solute is transferring. The constant c represents the intercept and includes information about the ratio of pseudostationary and aqueous buffer phases. The v term is a measure of the relative ease of forming a cavity for the solute in the aqueous buffer and pseudostationary phase. It is also a measure of hydrophobic interaction and related to the cohesiveness and dispersive properties of the pseudostationary phase. The coefficient e depends on the difference in ability of the pseudostationary phase and the buffer phase to interact with n- or π-electrons of solute while the s coefficient measures the dipolarity/polarizibility difference between the two phases. The coefficients a and b are the hydrogen bond accepting and hydrogen bond donating strengths of the pseudostationary phase, respectively. Through a comparative study of the five coefficients v, e, s, a, and b for the eight pseudostationary phases, their chemical selectivities can be determined.

Some reports on mixed surfactant systems have been published using LSER to understand the influence of the surfactant composition in MEKC [3,22]. Fuguet et al. analyzed 55 single, mixed and modified surfactant systems reported in the literature from over 200 pseudostationary phases characterized by LSER [3]. Among these selected surfactant systems, lithium perfluorooctane sulfonate (LPFOS), a fluorosurfactant with a C8 chain saturated with fluorine atoms, was found to have the most different selectivity with the extremely negative a and high b coefficients that are not found in any other systems. Based on the attractive properties of LPFOS, any of the mixtures containing it as a cosurfactant may show a wide variation of selectivities. However, sodium salt of LPFOS is insoluble, thus it cannot be mixed with SDS or other sodium surfactants; it can only be mixed with lithium surfactants. The influence of mixed micellar systems of SDS-sodium deoxycholate (SDC) and SDS-sodium cholate (SC) on retention and selectivity in MEKC were examined by Khaledi et al. using LSER [22]. In a comparative study of the LSER coefficients in the individual and mixed micellar systems, it was concluded by the authors that hydrogen bonding interactions had a significant effect on selectivity of the pseudostationary phases in MEKC. The interactive properties of the mixed micelles were found to be different from the constituent individual micelles; however, the overall characteristics were found to be closer to one of the bile salt micelles in the mixture even at the equimolar compositions.

In the present work, monomers of sodium 10-undecenyl sulfate (SUS), an achiral surfactant, sodium 10-undecenyl leucinate (SUL), a chiral surfactant, and their five binary mixtures were prepared and studied systematically. Their CMC, C_{20} (surfactant concentration that reduces the surface tension by 20 mN m^{-1}), γ_{CMC} (surface tension at CMC), partial specific volumes, methylene selectivity, mobilities, and the elution windows were determined using a variety of analytical techniques. They were then evaluated as pseudostationary phases in MEKC for separation of benzene derivatives with a wide range of chemical properties. As a commonly used pseudostationary phase in MEKC, SDS was also evaluated. To predict the selectivity differences between the eight surfactant systems, linear solvation energy relationships and free energy of transfer studies were conducted.

There are several objectives of this study. First, by changing the percent ratio of the two surfactants in their binary mixtures, the selectivity can be manipulated. Second, due to the protonation of carboxylate head group, amino acid-based chiral surfactants precipitate out of solution at acidic pHs, which limits their applications as pseudostationary phases. Their solubility can be significantly improved by combining them with highly soluble surfactants (e.g., SUS). The conformation and the charge density of the mixed micelles of SUL and SUS may vary at low and high pHs, which may affect the performance and selectivity of the mixed micelles. Lastly, the binary mixed micelles with carboxylate and sulfate head groups can be used as pH-responsive pseudostationary phases. McCarney et al. has recently used a pH-responsive polymer with sulfonate and carboxylate head groups, poly(sodium 2-(acrylamido)-2-methylpropanesulfonate)/11-(acrylamido)-undecanoic acid, (poly(NaAMPS/AmU)) [23]. At low pHs, the sulfonic acid groups in poly(NaAMPS/AmU) remain ionic whereas the carboxylate groups are not ionized. Both groups become ionized at higher pHs. Based on the static light scattering, quasi-elastic light scattering, viscometry, ^{1}H NMR spin–spin relaxation measurements, and fluorescence probe studies, it has been shown that the ionization of carboxylates changes the balance between ionic repulsion and hydrophobic interaction. As a result of this alteration, poly(NaAMPS/AmU) forms a compact conformation (unimer micelle) at acidic pHs and a more open configuration at basic pHs [24]. The change in conformation was found to affect the electrophoretic mobility, retention, selectivity, and separation efficiency. Higher electrophoretic mobility and greater affinity for majority of solutes were observed at lower pHs. In addition, very hydrophobic solutes with long alkyl chains were found to migrate with better efficiency at lower pHs.

These mixed micelles will be applied as chiral selectors for separation of chiral molecules at acidic and basic pHs and will be discussed in the subsequent parts of this series.

2. Experimental

2.1. Materials

All benzene derivatives, alkyl phenyl ketone homologues, *N*,*N*′-dicyclohexylcarbodiimide, L-leucine, chlorosulfonic acid, disodium hydrogenphosphate, sodium dihydrogenphosphate, and sodium hydroxide were obtained from Alfa Aesar (Ward Hill, MA, USA). *N*-hydroxysuccinimide and 10-undecen-1-ol were purchased from TCI America (Wellesley Hills, MA). Undecylenic acid and deionized water were obtained from Acros Organics (Morris Plains, NJ, USA), respectively and a water purification system from Millipore (Milford, MA, USA). All chemicals were used as received without further purification.

2.2. Preparation of sodium 10-undecenyl sulfate, 10-undecenyl L-leucinate and their binary mixtures

SUS and SUL were synthesized with minor modifications using procedures reported by Bergstrom and Lapidot, respectively, and reported elsewhere [25,26]. The following procedure was followed for preparation of the binary mixtures: 50 mM stock solutions of SUS and SUL were prepared separately. Given volumes of each

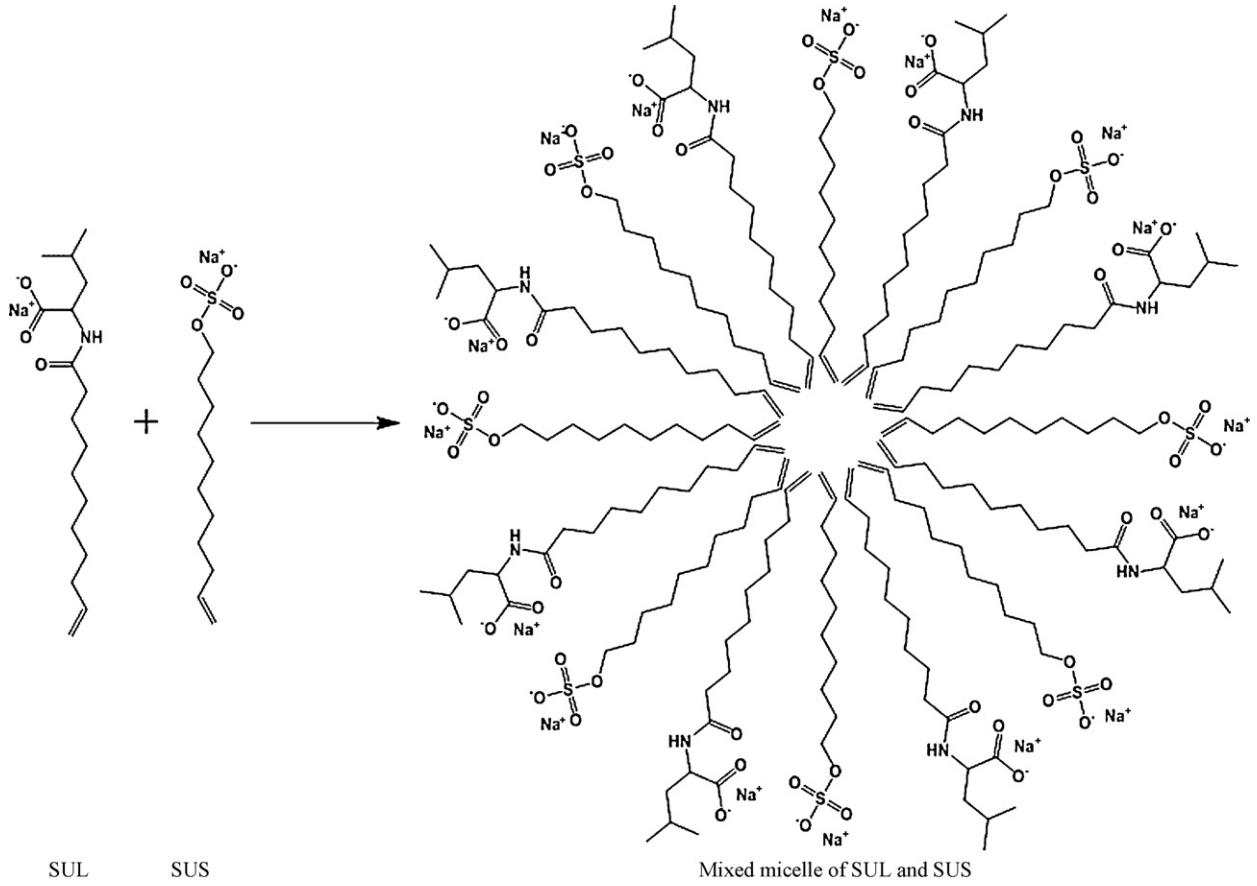

Fig. 1. Representative chemical structure of SUS–SUL binary mixture with 50:50 mole% fraction.

solution were mixed to prepare the desired mixture. For example, to prepare the 20:80 binary mixed surfactant, 2.0 mL SUS and 8.0 mL SUL solutions were mixed together. The first and the second numbers in the proposed acronyms (e.g., 20:80) for the binary mixtures represent the percent mole fractions of SUS and SUL (in SUS:SUL format), respectively. The total final concentration of the surfactants was kept at 50 mM and the mixture was sonicated thoroughly before use. A representative scheme of 50:50 binary mixed surfactant is presented in Fig. 1.

2.3. Characterization of surfactant systems

2.3.1. Determination of CMC, C_{20} and γ_{CMC}

Surface tension method was employed for CMC determination of SUS, SUL, their five binary mixtures, and SDS. A 50 mM stock solution of each of SUS and SUL was prepared in deionized water. Ten different concentrations ranging from 5.0 to 50.0 mM with 5.0 mM increments were prepared from the stock solution. A 20 mM stock solution of SDS was used to prepare several dilute concentrations for surface tension measurements. The following procedure was followed for binary mixtures: to prepare a binary mixture of 80:20 stock solution, 40 mL (80%) of SUS and 10 mL (20%) of SUL stock solutions were mixed to give a 50.0 mL mixed surfactant solution. The other binary mixtures were prepared similarly. These stock solutions were then diluted to prepare a series of concentrations ranging from 5.0 to 50.0 mM with 5.0 mM increments. The surface tension measurements were taken by a KSV Sigma 703D digital tensiometer (Monroe, CT, USA) using a DuNoüy ring. Surface tension values were plotted against surfactant concentration and the CMC was taken as the breakpoint of the curve of surface tension versus concentration. The γ_{CMC} and C_{20} values were also determined from the same curve. All measurements were repeated at least 3 times at ambient temperature.

2.3.2. Determination of partial specific volume

An approach based on the density (ρ) measurement of the surfactant solution was used for partial specific volume, $\bar{v}$, determination [27]. A graph of $1/\rho$ against weight fraction of solvent (W, weight of solvent/weight of solution) allows the determination of partial specific volume from the value of the y-intercept. The solutions for density measurements were prepared in a similar way as those for CMC measurements. A 50 mM stock solution of each surfactant system was prepared in deionized water. About 3 mL of five different concentrations ranging from 10.0 to 50.0 mM with 10.0 mM increments were prepared from the stock solutions. The densities were measured at 25 °C using a high-precision digital DMA 4500 density meter (Anton Paar, Ashland, VA, USA). The calibration of the density meter was done with dry air and deionized water at 25 °C.

2.4. Capillary electrophoretic separations

2.4.1. Instrumentation

An Agilent CE system (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector was used for MEKC separations. The system control and data handling were done using 3D-CE ChemStation software. The MEKC separations were performed in fused-silica capillaries (Polymicro Technologies, Tucson, AZ, USA) with dimensions of 66.0 cm total length (57.5 cm effective length) × 50 μm ID (360 μm OD). Capillaries used in this study were cut from the same capillary bundle and were reactivated thoroughly after each surfactant system using deionized water (10 min)

and 1.0 M NaOH (ca. 20 min) to eliminate possible cross contaminations.

2.4.2. Preparation of separation buffers and solute solutions

A 40 mM stock solution of each of SUS and SUL was prepared in 10 mM phosphate buffer (pH 7.0). The binary mixtures were prepared by mixing given volumes of each surfactant solution. For example, to prepare a 50:50 mixed surfactant, 2.5 mL SUS solution and 2.5 mL SUL solution were mixed. Each surfactant solution was sonicated for 5 min, filtered through a 0.45-μm syringe filter (Nalgene, Rochester, NY, USA), and degassed for one additional min before use in MEKC experiments. Stock solutions of benzene derivatives were prepared in methanol and diluted with 50:50 methanol:deionized water before injection.

2.4.3. Micellar electrokinetic chromatography of benzene derivatives

Each new capillary was activated with 1 M NaOH (30 min at 40 °C) and deionized water (10 min at 25 °C) before use. For a typical MEKC run, the capillary was rinsed for 3 min with triply deionized water and for 3 min with 0.1 M NaOH followed by a 3 min rinse with separation buffer between injections. Each day, the capillary was reactivated by rinsing with 1 M NaOH (10 min) and triply deionized water (5 min). Unless otherwise noted, the applied voltage was +30 kV and the injection size was 50 mbar for 1 s. Peaks were identified by comparison of their individual UV-spectrum obtained from diode array detector or via spiking when necessary.

2.5. Calculations

The capacity factor values, k, of neutral solutes were calculated by use of the following equation [28]:

$$k = \frac{t_R - t_{eof}}{t_{eof}\left[1 - \left(t_R/t_{psp}\right)\right]} \quad (2)$$

where t_R, t_{eof} and t_{psp} are the migration-times of solute, electroosmotic flow (eof), and the pseudostationary phase, respectively. Methanol and undecanophenone were used to measure t_{eof} and t_{psp} markers, respectively.

By graphing $\log k$ versus carbon number of nine alkyl phenyl ketones (i.e., acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, decanophenone and undecanophenone; the last two generally coeluted and used as pseudostationary phase marker), the methylene selectivity (also called hydrophobic selectivity), α_{CH_2}, was calculated from the antilogarithm of the slope of the trend line. The electroosmotic mobility of buffer solution, μ_{eo}, was calculated by use of Eq. (3):

$$\mu_{eo} = \frac{I_t I_d}{V t_{eof}} \quad (3)$$

where I_t is the total length of capillary (cm), I_d is the length of capillary from injector to detector (cm), and V is the applied voltage (V). The retention times were measured in s. To calculate the apparent electrophoretic mobility of pseudostationary phases, μ_{app}, the t_{eof} term in Eq. (3) was replaced with t_{psp}. The effective electrophoretic mobilities of pseudostationary phases (μ_{ep}) were calculated by taking the difference between μ_{eo} and μ_{app} ($\mu_{ep} = \mu_{app} - \mu_{eo}$). The k is related to distribution coefficient, K, by the following equation:

$$K = \frac{k}{\bar{\nu}([S_{tot}] - \mathrm{CMC})} \quad (4)$$

where, $\bar{\nu}$ is the partial specific volume and $[S_{tot}]$ is the total concentration of the pseudostationary phase. The phase ratio of the surfactant system, β, can be determined using the following equation [28,29]:

$$\beta = \frac{V_{psp}}{V_{aq}} = \frac{\bar{\nu}([S_{tot}] - \mathrm{CMC})}{1 - \bar{\nu}([S_{tot}] - \mathrm{CMC})} \quad (5)$$

where V_{psp} and V_{aq} are the volume of the pseudostationary and aqueous phase, respectively. Finally, the elution window was calculated using t_{psp}/t_{eof} ratio. The system coefficients, v, e, s, a, and b, described in Eq. (1) are determined by multiple linear regression using SAS software (SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1. Characterization of pseudostationary phases

3.1.1. CMC, C_{20} and γ_{CMC} comparison

The CMC values and other physicochemical properties of the pseudostationary phases are listed in Table 1. Surface tension is a measure of the surface activity of a solution. The surface tension measurement is a classical method of studying the CMC of surfactants. This method is based on the surfactant concentration dependence of surface tension. It decreases as the concentration of surfactant is increased and levels off at a certain concentration. The CMC values of SUS and SUL were found to be 26 and 16 mM, respectively, while those of mixed surfactants were found to be very similar to that of SUL. As compared with sulfate head group, leucinate is more hydrophobic due to its isobutyl side chain which is believed to be responsible for decreased ionic repulsion between charged head groups, thus, resulting in aggregation of SUL monomers at lower concentrations. The CMC of SDS is nearly 3 times lower than that of SUS due to the longer hydrophobic carbon tail of SDS (C12 in SDS versus C11 in SUS). The presence of a double bond at the end of carbon chain makes the SUS micelles less hydrophobic than SDS. An increase in hydrophobicity (i.e., addition of extra CH_2 groups or lack of double bond in carbon chain) lowers the CMC and thus favors formation of the micelles at lower concentrations [30].

The C_{20} and γ_{CMC} represent the surfactant concentration in the solution phase that reduces the surface tension of the solvent by 20 mN m^{-1} and the surface tension at CMC, respectively. Since these two properties are related to the surface activity of the surfactants, their values are also reported in this study. These properties were determined from the surface tension versus surfactant concentration graphs (not shown) and are listed in Table 1. The C_{20} for SUL (0.13 mM) found to be smaller by over two orders of magnitude than that for SUS (15.64 mM) shows the excellent efficiency of SUL at reducing the surface tension. The C_{20} values decreased dramatically as the percent mole fraction of SUL was increased in the mixed micelles. The C_{20} for SDS is about 5 times lower than that of SUS due probably to the higher hydrophobicity of SDS but about 24 times higher than that for SUL. Among the pseudostationary phases studied, SDS and SUS showed the highest and the lowest surface activity. The surface activities of SUL and the binary mixed surfactants were found to be very similar.

3.1.2. Partial specific volume, phase ratio and methylene group selectivity comparison

Partial specific volume is defined as an increase in the volume upon dissolving 1.0 g of a dry material in a large volume of a solvent at constant temperature and pressure. To determine the partial specific volume, change in volume of the solvent needs to be accurately measured upon addition of infinitesimal amount of surfactant. Since the measurement of such a small volume change is very difficult, the partial specific volume can be determined from density values.

Partial specific volume is a thermodynamic parameter and is sensitive to various intermolecular interactions (e.g., hydropho-

Table 1
Physicochemical properties of the surfactant systems used in present study.

Physicochemical property	Pseudostationary phase							
	SUS	80:20	60:40	50:50	40:60	20:80	SUL	SDS
CMC[a] (mM)	26	18	17	16	17	16	16	8
C_{20}[b] (mM)	15.64	3.99	2.22	2.11	1.19	0.16	0.13	3.2
γ_{CMC}[c] (mN m^{-1})	41.6	37.1	35.7	36.3	36.9	36.1	37.6	33.2
Partial specific volume[d], $\bar{\nu}$ (mL g^{-1})	0.800	0.945	0.844	0.843	0.860	0.875	0.874	0.853
Phase ratio[e], β	0.0196	0.0212	0.0198	0.0206	0.0202	0.0215	0.0306	0.0103
Electroosmotic mobility[f], μ_{eo} (10^{-4} cm^2 V^{-1} s^{-1})	4.51	5.48	5.59	5.79	5.86	5.96	5.98	6.27
Apparent electrophoretic mobility[f,g], μ_{app} (10^{-4} cm^2 V^{-1} s^{-1})	0.71	1.58	1.74	1.94	2.03	2.07	2.16	1.95
Effective electrophoretic mobility[f,g], μ_{ep} (10^{-4} cm^2 V^{-1} s^{-1})	−3.80	−3.89	−3.85	−3.85	−3.83	−3.88	−3.81	−4.31
Methylene group selectivity[f,h], α_{CH_2}	2.38	2.31	2.38	2.41	2.45	2.47	2.50	2.61
Migration-time window[f], t_{psp}/t_{eof}	6.4	3.5	3.2	3.0	2.9	2.9	2.8	3.2

[a] Critical micelle concentration; determined by surface tensiometer.
[b] Surfactant concentration that reduces the surface tension by 20 mN m^{-1}; determined by surface tensiometer.
[c] Surface tension at CMC; determined by surface tensiometer.
[d] Determined by density meter.
[e] Eq. (5) was used for phase ratio calculation.
[f] Data were collected with 60 cm (57.5 cm effective length) × 50 μm ID capillary with an applied voltage of +30 kV using a 10 mM phosphate buffer at pH of 7.0; temperature, 25 °C; final surfactant concentration: SDS, 40 mM; SUS and SUL, 50 mM; mixed surfactants, 40 mM (total concentration).
[g] μ_{app}, μ_{eo}, and μ_{ep} were calculated using Eq. (3) and related equations given in the text.
[h] Calculated from the antilogarithm of the slope of the regression line of log k versus carbon number of alkyl phenyl ketones [C8 (acetophenone) – C14 (octanophenone)].

bic and hydrophilic interactions) involving solute (e.g., surfactant) and solvent (e.g., water). In other words, partial specific volume is closely related to hydration of the micelles [31,32]. An increase in partial specific volume can be attributed to hydration of the hydrophilic outer layer of the micelle. Similarly, a decrease in partial specific volume may be due to the dehydration of the micelle, which results in a relatively more compact micelle [33,34]. As listed in Table 1, SUS has the lowest (0.800 cm^3/g) and SUL has the largest partial specific volume (0.874 cm^3/g). Compared with SUS, SDS has a larger value (0.853 cm^3/g) owing probably to its longer carbon chain. The partial specific volumes of 60:40 and 50:50 mixed micelles are similar. The values increase from 0.843 to 0.875 cm^3/g upon an increase in SUL content from 50% to 80%. It is worth noting that 80:20 has the largest partial specific volume (0.945 cm^3/g) among the surfactant systems studied. The rationale behind this unexpectedly high value is not clear but it is believed that the water molecules at the outer layer of the micelle make 80:20 mixed surfactant less hydrophobic. This assertion is also supported by the methylene group selectivity data (see below).

The phase ratios, β, of the surfactant systems are listed in Table 1. As expressed from Eq. (5), the phase ratio is related to the partial specific volume, total concentration and the CMC of the surfactant in electrolyte system. Under the experimental conditions used in this study, SDS and SUL systems have the lowest (0.0103) and the highest (0.0306) phase ratios, respectively. The addition of SUL to SUS increased the phase ratio to some extent. The phase ratios for the synthetic surfactants (e.g., SUS, SUL and the mixed surfactant systems) are not available in the literature for comparison purposes, however, that of SDS determined in this study (0.0103) is comparable with the literature value of 0.0105 [35].

The α_{CH_2} values are also listed in Table 1. With its longest carbon chain, SDS provides the most hydrophobic environment (highest value of 2.61). The second most hydrophobic surfactant is SUL ($\alpha_{CH_2} = 2.50$) due probably to the presence of leucinate head group whereas SUS, with its unsaturated carbon tail, is among the least hydrophobic surfactants ($\alpha_{CH_2} = 2.38$). The introduction of relatively hydrophobic SUL into the mixture is expected to increase the hydrophobicity of the binary mixed surfactant, but addition of initial 20% of SUL resulted in a mixed surfactant (80:20) with the least hydrophobicity ($\alpha_{CH_2} = 2.31$), which makes this binary mixed surfactant even less hydrophobic than SUS. Further increase in mole fraction of SUL gradually increased the α_{CH_2} making the remaining mixed surfactants more hydrophobic as compared with 80:20 mixed surfactant.

3.1.3. Mobilities and migration-time window comparison

The electroosmotic mobility, μ_{eo}, apparent electrophoretic mobility, μ_{app}, effective electrophoretic mobility, μ_{ep}, and the migration-time window, t_{psp}/t_{eo}, values for the pseudostationary phases are listed in Table 1. The SUS and SDS (both are sulfated surfactants) have the lowest (4.51×10^{-4}) and the highest (6.27×10^{-4}) μ_{eo} values, respectively; whereas SUL has a value (5.98×10^{-4}) similar to that of SDS. Addition of 20% SUL to SUS solution increased the μ_{eo} value to 5.48×10^{-4}. A steady increase in μ_{eo} values was observed as the percent mole fraction of SUL was further increased from 20 to 80. These variations in the μ_{eo} for different surfactant systems can be attributed to a variety of parameters including viscosity of the surfactant solution, zeta potential of both capillary walls and pseudostationary phases, and the charge density on the capillary wall upon addition of the surfactants. The μ_{app} values gradually increased as the content of SUL was increased from 20% to 80%. Anionic pseudostationary phases have negative μ_{ep} values because they are attracted to the anode (the opposite direction of eof movement). However, because the μ_{eo} is greater than the μ_{ep}, the stronger eof drags the surfactants toward the cathode. The mixed micelles had practically the same μ_{ep} values. The migration-time window was the widest for SUS and, taking the standard error into account, the remaining surfactant systems had almost the same migration-time window.

All physicochemical properties are summarized in Fig. 2. A correlation was found between the migration-time window and the γ_{CMC} values. Since surface tension is related to the activity of micellar phase, this relationship is not surprising. Inverse relationship between γ_{CMC}, apparent electrophoretic and electroosmotic mobilities is interesting to note. Correlation among phase ratio, γ_{CMC} of the surfactant systems and their electrophoretic mobilities in MEKC is also visible with the exception of SUL system. The inverse relationship between methylene group selectivity and partial specific volume it is also worth noting (Fig. 2, inset).

3.2. Electrokinetic separation of benzene derivatives and distribution coefficients

Solute interactions with the pseudostationary phases occur via a number of mechanisms such as surface adsorption, coaggregation, or partitioning into the hydrophobic core of the micelles. Thus, depending upon their physicochemical nature, analyte may reside in several regions of the micelle. For example, hydrophilic solutes reside near the polar head group while the hydrophobic

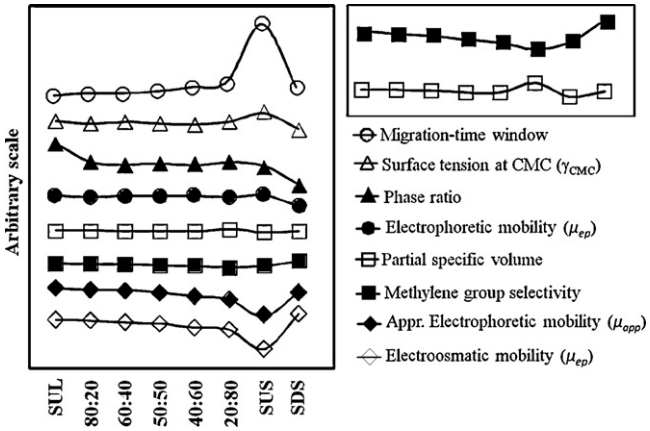

Fig. 2. Comparison of physicochemical properties. The expansion of partial specific volume and methylene group selectivity as well as the legends are shown on the right side of the graph.

ones can penetrate into the hydrophobic micellar core. Solutes with amphiphilic character have special interaction with the micelle and align themselves with the nonpolar part of the analyte directed toward the hydrophobic core and the polar part directed to the bulk aqueous phase. As a result of these different mechanisms, the retention of the analytes in each pseudostationary phase system is expected to be different.

To understand the mechanisms of solute interaction with the surfactants systems studied, the retention behavior of 29 benzene derivatives with diverse properties is studied. The benzene derivatives used in this study are characterized as non-hydrogen bond donors (NHBs), hydrogen bond acceptors (HBAs), and hydrogen bond donors (HBDs). The NHB solutes include alkyl- and halo-substituted benzenes and polycyclic aromatic hydrocarbons (e.g., naphthalene) and do not hold any hydrogen bonding functional groups; however, due to the aromatic ring(s), they are considered to be weak hydrogen bond acceptors. The HBAs possess only hydrogen bond accepting functional groups on the aromatic ring, whereas, the HBDs have both hydrogen bond donating and hydrogen bond accepting functional groups. Based on their pK_a values, all test solutes are believed to be neutral at pH 7.0.

The partitioning coefficient values, K, are calculated using Eq. (4) and are compared in Fig. 3A–C. The K values of the first six NHBs (benzene, toluene, chlorobenzene, bromobenzene, ethylbenzene, and p-xylene) are very similar in all surfactant systems (Fig. 3A) while those of relatively hydrophobic solutes (iodobenzene, 4-chlorotoluene, naphthalene, and propylbenzene) are dissimilar in each surfactant system. The K values of the hydrophobic NHBs are relatively lower in 80:20 and 20:80 mixed surfactants, which have similar partial specific volume and phase ratios. The HBA solutes produce relatively smaller K values as compared with the NHBs.

Electropherograms of the benzene derivatives using eight pseudostationary phases were compared (data not shown). The SUS and SUL were found to provide the widest (ca. 13.3 min) and the narrowest (ca. 5.1 min) elution windows (i.e., difference in retention times of the last and the first eluting solutes) for NHB solutes. The rest of the surfactant systems provided elution windows ranging from 5.3 (20:80) to 7.3 min (80:20). Similar trend was observed for HBA analytes but the elution windows were relatively narrower. As compared with NHB and HBA solutes, the elution window for HBD solutes was even narrower. It is worth mentioning that the elution window between the first eluting alkyl phenyl ketone (i.e., acetophenone) and the last one (i.e., undecanophenone) became narrower as the content of SUL was increased.

Strength of the interaction between pseudostationary phases and the solutes can be, in general, ordered as: NHB > HBA > HBD. This retention behavior can be attributed to the major role of the hydrophobic interactions on solute retention in MEKC. Although there were some minor resolution differences between adjacent peaks, the migration order of the solutes was the same for the NHB analytes in all pseudostationary phases. 4-Chloroanisole eluted last in SUL, 80:20, 60:40 and 50:50 surfactant systems but co-eluted with ethyl benzoate in 40:60 and eluted before ethyl benzoate in 20:80, SUS and SDS surfactant systems (Fig. 3B). As seen in Fig. 3C, pseudostationary phases show the most diverse selectivity towards HBD analytes. For example, benzyl alcohol and phenol coelute in SUS but are separated in the remaining surfactant systems. In addition, the last two compounds (i.e., 3-bromophenol and 4-bromophenol) coelute in all surfactant systems except in 80:20 and SUL where they are partially separated.

3.3. LSER results

There are a few important requirements that should be fulfilled for successful application of the LSER to characterization of pseudostationary phases in MEKC [13]. First, statistically sufficient number of compounds must be used to have a statistically sound data. Second, for a wide range of interaction, a diverse set of solutes with diverse properties such as NHB, HBA and HBD must be used. Third, many solutes, particularly those in a homologous series, have very similar descriptor values, which can result in determination of the system constants with low accuracy; thus, no significant cross-correlations must exist between the descriptors.

The benzene derivatives used in this study and their descriptors are listed in Table 2 (solute descriptors are from ref. [36]). A cross-correlation matrix of these solutes showed no correlation between the solute descriptors (Table 3). The system coefficients c, v, e, s, a, and b were calculated by substituting the log k and the solute descriptor values into Eq. (1) using multiple linear regression. The LSER provided acceptable but relatively poor statistics due to the outliers (Table 4, top). The F values ranged from 78 to 246 and correlations, R^2, values ranged from 0.945 to 0.982. The outliers were determined by their standardized residual values (data not shown); residuals greater than 2 were removed from the list. Each surfactant system was found to have at least two outliers (except SDS). 4-Fluorophenol was an outlier in all surfactant systems (except SDS). The other outliers were 3-methylphenol (in all except SDS, SUS and SUL), propiophenone (in SUS and 80:20), toluene (in 20:80), ethylbenzene (in SUS), benzonitrile (in 60:40), and 4-chloroaniline (in SUL). After the removal of these outliers, the system constants were recalculated and are listed in Table 4 (bottom). Removal of the outliers improved the statistics significantly ($F \approx 148$–444; $R^2 \approx 0.974$–0.991).

Before interpreting the LSER data, it is helpful to remember that the coefficients are related to the properties of the separation system. These coefficients reflect differences in the two phases (i.e., micellar and aqueous buffer phase). Large coefficients indicate large differences while small or statistically insignificant coefficients indicate no difference between the two phases. Furthermore, the sign of the coefficient indicates whether the aqueous or the pseudostationary phase interacts more strongly with the solute.

3.3.1. System constant c

As seen in Table 4, the value of the c constant (or LSER intercept) decreases, in general, as the percent mole fraction of SUL is increased in the mixed surfactant systems. It is interesting to note that the surfactant with the lowest phase ratio (SUS, 0.0196) has the lowest (or most negative) c constant value (−2.97) and the one with the highest phase ratio (SUL, 0.0306) is among the surfactants with the highest (least negative) c constant values (−2.53). The relationship between these two parameters can be attributed to the fact that the c constant contains the phase ratio (i.e., the ratio

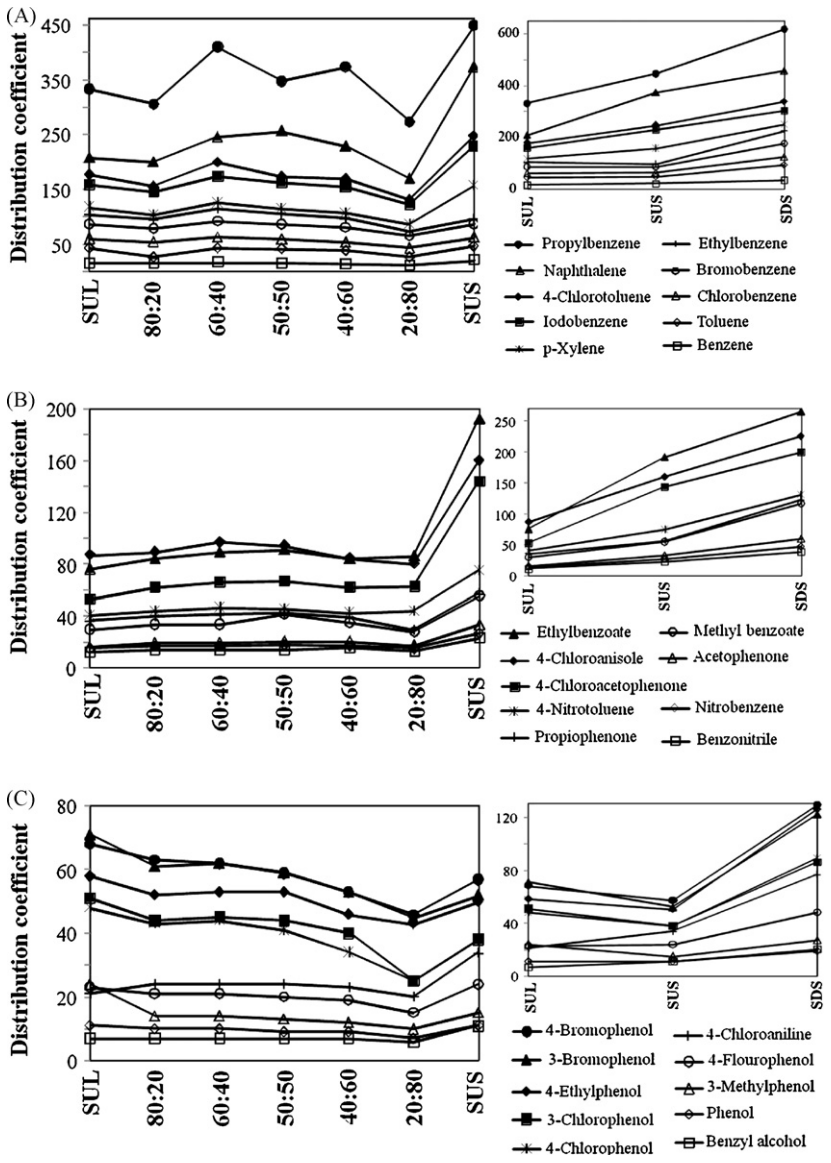

Fig. 3. Distribution coefficient of (A) NHB, (B) HBA and (C) HBD solutes as a function of surfactant composition. Inset: comparison of SUL, SUS and SDS surfactant systems. Legends are shown in the figure.

of the pseudostationary phase volume to the aqueous phase volume) of the separation system. Since it contains some other system off-sets together with the phase ratio, the *c* constant is not always well-correlated with the phase ratio, as seen in the case of SDS system, which has the lowest phase ratio and highest *c* constant. It is important to note that the intercept contains helpful chemical information but its interpretation is difficult because of its complex nature [37].

3.3.2. The effect of surfactant composition on cohesiveness

The v coefficient is positive and has the largest values in all the pseudostationary phases (Table 4). The magnitude of v is related to the difference in cohesive energies of the aqueous phase and the micellar phase; it indicates the greatest influence of cohesiveness on MEKC retention. In other words, it is a measure of the relative ease of forming a cavity for the solute in the two phases. The larger the v value the smaller the cohesive energy of the micellar phase. The positive sign indicates that the micellar phase is less cohesive (more hydrocarbon-like) than aqueous phase, thus, the hydrophobic solutes prefer to transfer to micellar phase. As seen in Table 4, the v coefficient values range from 3.04 (20:80) to 3.30 (SUS). Although the difference in v coefficient is not immense, the combination of SUL and SUS does affect the cohesiveness of the mixed micelles. Among surfactant systems studied, SUS provides the most hydrocarbon-like and 20:80 mixed micelle provides the least hydrocarbon-like environment for the solutes. In general, an increase in SUL content produces more hydrophobic (or hydrocarbon-like) mixed micelles. The v coefficient value (as well as other coefficients) for SDS obtained in this study is very similar to the values for SDS reported in the literature [13,38]. Based on the methylene group selectivity values (Table 1), which were determined using a number of alkyl phenyl ketones, SDS is the most hydrocarbon-like surfactant ($\alpha_{CH_2} = 2.61$) due to its longer hydrophobic tail as compared with SUS ($\alpha_{CH_2} = 2.38$). In accordance with the v coefficient values, SUS is more hydrocarbon-like surfactant than SDS. This discrepancy might be attributed to water molecules penetrated into the micelles [37]. Since the NHB, HBA and HBD benzene derivatives generally reside in the palisade and the Stern layer of the micelles, they experience the presence of the water molecules in those regions more than the alkyl phenyl ketones do. As a result, the LSER, which utilizes the NHB, HBA and HBD solutes, and the methylene selectivity, which uti-

Table 2
Test solutes and their solvation descriptors.[a].

No	Solutes	Solute descriptors				
		V	*E*	*S*	*A*	*B*
NHB solutes						
1	Benzene	0.716	0.610	0.52	0.00	0.14
2	Toluene	0.857	0.601	0.52	0.00	0.14
3	Chlorobenzene	0.839	0.718	0.65	0.00	0.07
4	Bromobenzene	0.891	0.882	0.73	0.00	0.09
5	Ethylbenzene	0.998	0.613	0.51	0.00	0.15
6	p-Xylene	0.998	0.613	0.52	0.00	0.16
7	Iodobenzene	0.975	1.188	0.83	0.00	0.12
8	4-Chlorotoluene	0.980	0.705	0.67	0.00	0.07
9	Naphthalene	1.085	1.360	0.92	0.00	0.20
10	Propylbenzene	1.139	0.604	0.50	0.00	0.15
HBA solutes						
11	Benzonitrile	0.8710	0.742	1.11	0.00	0.33
12	Nitrobenzene	0.8910	0.871	1.11	0.00	0.28
13	Acetophenone	1.0140	0.818	1.01	0.00	0.48
14	Methyl benzoate	1.0730	0.733	0.85	0.00	0.46
15	Propiophenone	1.1550	0.800	0.95	0.00	0.51
16	4-Nitrotoluene	1.0320	0.870	1.11	0.00	0.28
17	4-Chloroacetophenone	1.1360	0.955	1.09	0.00	0.44
18	4-Chloroanisole	1.0380	0.838	0.86	0.00	0.24
19	Ethyl benzoate	1.2140	0.689	0.85	0.00	0.46
HBD solutes						
20	Benzyl alcohol	0.9160	0.803	0.87	0.33	0.56
21	Phenol	0.7750	0.805	0.89	0.60	0.30
22	3-Methylphenol	0.9160	0.822	0.88	0.57	0.34
23	4-Flourophenol	0.7930	0.670	0.97	0.63	0.23
24	4-Chloroaniline	0.9390	1.060	1.13	0.30	0.31
25	4-Chlorophenol	0.8980	0.915	1.08	0.67	0.20
26	3-Chlorophenol	0.8980	0.909	1.06	0.69	0.15
27	4-Ethylphenol	1.0570	0.800	0.90	0.55	0.36
28	3-Bromophenol	0.9500	1.060	1.15	0.70	0.16
29	4-Bromophenol	0.9500	1.080	1.17	0.67	0.20

[a] Solute descriptors from [36].

lizes alkyl phenyl ketones, provide contradictory hydrophobicity data.

3.3.3. The effect of surfactant composition on hydrogen bonding

The *b* coefficient has the second largest magnitude signifying the strong influence of hydrogen bonding in MEKC retention and selectivity. The negative sign of the *b* coefficient indicates that the micellar phase is less acidic (i.e., has poor hydrogen bond donating ability) than the aqueous phase because water molecules in aqueous phase are more capable of donating hydrogen bonds. The *b* coefficient values vary from −2.37 (SUL) to −1.88 (SDS). Surfactants with sulfate head group (i.e., SUS and SDS) have the least negative *b* coefficient values; thus, they are the strongest hydrogen bond donors ability among the micellar phases studied. In contrast, SUL with leucinate head group is the weakest hydrogen bond donor (*b* coefficient = −2.37). Surfactants with sulfate and leucinate head group are expected to show hydrogen bond accepting capacity due to the presence of oxygen and nitrogen atoms, however, the reverse is observed. It has been suggested that the water molecules in the palisade and Stern layers of the micelles are responsible for the hydrogen bonding properties of the micelles. Water can penetrate as far as the second or third methylene unit (from the head group)

Table 3
Cross-correlation matrix of the descriptors of the 29 solutes.

	V	*E*	*S*	*A*	*B*
V	1.0000				
E	0.0198	1.0000			
S	0.0038	0.3784	1.0000		
A	0.1233	0.0555	0.2131	1.0000	
B	0.1715	0.0002	0.1658	0.0002	1.0000

Table 4
System constant and regression statistic for the investigated surfactant systems.

Coefficients	Surfactant systems							
	SUS	80:20	60:40	50:50	40:60	20:80	SUL	SDS
Before removal of outliers								
c	−2.82 (±0.21)	−2.67 (±0.17)	−2.54 (±0.15)	−2.52 (±0.15)	−2.50 (±0.15)	−2.59 (±0.16)	−2.46 (±0.11)	−2.25 (±0.11)
v	3.12 (±0.22)	3.13 (±0.18)	3.10 (±0.16)	3.10 (±0.16)	3.17 (±0.16)	3.08 (±0.17)	3.09 (±0.12)	3.16 (±0.12)
e	0.34 (±0.17)*	0.26 (±0.13)*	0.31 (±0.12)	0.35 (±0.12)	0.31 (±0.12)	0.28 (±0.13)	0.32 (±0.09)	0.12 (±0.09)*
s	−0.22 (±0.17)*	−0.22 (±0.14)*	−0.33 (±0.12)*	−0.36 (±0.12)	−0.38 (±0.12)	−0.26 (±0.13)*	−0.46 (±0.09)	−0.01 (±0.09)*
a	−0.29 (±0.09)	−0.11 (±0.07)*	−0.05 (±0.07)*	−0.02 (±0.07)*	0.01 (±0.07)*	0.05 (±0.07)*	0.20 (±0.05)	−0.19 (±0.05)*
b	−1.98 (±0.21)	−2.21 (±0.17)	−2.29 (±0.15)	−2.28 (±0.15)	−2.44 (±0.15)	−2.28 (±0.16)	−2.41 (±0.11)	−1.76 (±0.11)
F[a]	78	116	143	147	161	123	246	217
R^2[b]	0.945	0.962	0.969	0.970	0.972	0.964	0.982	0.961
n	29	29	29	29	29	29	29	29
After removal of outliers								
c	−2.97 (±0.16)	−2.77 (±0.11)	−2.64 (±0.09)	−2.56 (±0.10)	−2.55 (±0.10)	−2.52 (±0.10)	−2.53 (±0.09)	−2.25 (±0.11)
v	3.30 (±0.17)	3.21 (±0.12)	3.19 (±0.10)	3.12 (±0.11)	3.19 (±0.11)	3.04 (±0.10)	3.10 (±0.09)	3.16 (±0.12)
e	0.43 (±0.13)	0.37 (±0.09)	0.49 (±0.08)	0.45 (±0.08)	0.42 (±0.08)	0.37 (±0.08)	0.41 (±0.07)	0.12 (±0.09)*
s	−0.33 (±0.13)	−0.34 (±0.09)	−0.55 (±0.09)	−0.48 (±0.08)	−0.49 (±0.08)	−0.41 (±0.08)	−0.49 (±0.07)	−0.01 (±0.09)*
a	−0.33 (±0.07)	−0.09 (±0.05)*	0.01 (±0.05)*	0.01 (±0.05)*	0.03 (±0.05)*	0.06 (±0.04)*	0.17 (±0.04)	−0.19 (±0.05)*
b	−1.90 (±0.15)	−2.04 (±0.11)	−2.15 (±0.09)	−2.16 (±0.10)	−2.33 (±0.10)	−2.17 (±0.09)	−2.37 (±0.08)	−1.76 (±0.11)
F	148	243	350	304	323	327	444	217
R^2	0.974	0.984	0.989	0.986	0.987	0.988	0.991	0.961
n	26	26	26	27	27	26	27	29

[a] Fisher's test.
[b] Correlation coefficient of linear regression.
* Values are not statistically significant at the 95% confidence level.

of the surfactant tail in a micelle [8,39–41]. Thus, the hydrogen bond capacity of the pseudostationary phases is mostly due to the amount, orientation, attachment, and penetration of water in the micelle.

The *a* coefficient represents the difference in hydrogen bond accepting ability (basicity) of the pseudostationary phase and that of the aqueous phase. It is one of the least significant factors in LSER for the surfactant systems studied. Only three surfactants (i.e., SUS, SDS, and SUL) provide statistically significant but relatively very small *a* coefficients. As verified by their negative *a* coefficients, SUS and SDS are the least basic (i.e., have the weakest hydrogen bond accepting ability) surfactants. SUL, the only surfactant with a positive *a* coefficient value, is more basic than the aqueous phase. The hydrogen basicity of SUL can be attributed to the presence of –NH group in the leucinate head group besides the water molecules in the micelle. It is worth noting that, although both have sulfate head group, SDS is relatively better hydrogen bond accepting than SUS.

3.3.4. The effect of surfactant composition on dipolarity and polarizability

The coefficient *e* is related to the ability of the pseudostationary phase to interact with *n*- or π-electrons of the solutes (polarizability/excess molar refraction) and the *s* coefficient is related to dipolarity/polarizability of the pseudostationary phase. Both of the coefficients are small in magnitude for all surfactant systems, indicating a small influence of these parameters on selectivity. As seen in Table 4, the sign of *e* and *s* coefficients are positive and negative, respectively. The positive sign of the *e* coefficient indicates that the surfactant systems possess higher degree of polarizability than the aqueous phase and can interact with or become polarized by *n*- and π-electrons of the solutes. It should be noted that the hydrophilic head groups of the individual and the mixed surfactant systems possess easily polarizable moieties, i.e., carbon–oxygen or sulfur–oxygen double bonds. The value of *e* coefficient ranges from 0.29 (SDS) to 0.49 (60:40 mixed micelle). The 60:40 mixed surfactant and SDS are the most and the least polarizable phases, respectively. This can be attributed to the fact that at 50 and 60 mole% fraction of SUS, the micelles take a unique conformation so that the easily polarizable moieties (e.g., C=O and S=O double bonds) are more accessible to the solutes for better interaction. In SDS micelles, however, this accessibility is believed to be limited by the water molecules in the palisade layer of the micelles. Among the surfactant systems studied, SDS has the longest hydrocarbon tail (C12). Spectroscopic experiments have shown that chain length of the surfactant influences the depth of water penetration into the micelle [42–44]. Surface tension studies have also suggested that the amount of water in the micelle increases with increasing the chain length [45]. The negative *s* coefficient values indicate that all pseudostationary phases are less dipolar than the aqueous phase. SDS and SUS are the most dipolar whereas SUL and 60:40 mixed surfactants are the least dipolar phases.

Comparison of the system coefficients for the pseudostationary phases as a function of surfactant composition is summarized in Fig. 4. As seen from the plot, a trend in the values of some of the coefficients as a factor of SUS content is apparent. For example, an increase in SUS concentration results in a decrease the *c* constant. Similar trend is also observed in *a* constant. A correlation is observed between *v* and *e* coefficients. The inverse relationship between s and e coefficients is also apparent (Fig. 4, inset).

3.4. The effect of surfactant composition on free energy of transfer

The mixed and individual pseudostationary phases can be further characterized by evaluating the differences in free energy of transfer for different functional groups on benzene ring. The

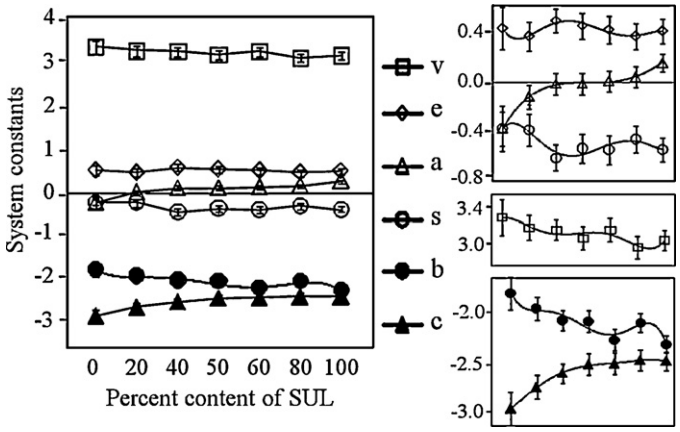

Fig. 4. Plot of the system constants derived from the LSER against percent content of SUL, where 0 and 100 on *x*-axis represent SUS and SUL, respectively. The legends and expended plots are shown on the right side of the plot.

functional group selectivity, τ, can be defined as the ratio of capacity factor of a substituted benzene (k_{Bz-R}) to that of benzene (k_{Bz}): $\tau = k_{Bz-R}/k_{Bz}$ [15]. The τ value can then be used to determine the difference in free energy of transfer, $\Delta\Delta G$, of a functional group from aqueous buffer phase to the micellar phase using the following equation: $\Delta\Delta G = -RT\ln\tau$, where R is the universal gas constant (8.314 J/mole) and T is the temperature (K). The change in free energy of transfer for a functional group is related to the changes in free energies due to the cavity formation (the *v* coefficient), hydrogen bonding (the *a* and *b* coefficients), dipolarity (the *s* coefficient) and the polarizability (the *e* coefficient) [46]. The $\Delta\Delta G$ values for various functional groups are presented in Fig. 5. A negative $\Delta\Delta G$ value indicates that the addition of a functional group to benzene ring leads to an increase in strength of the interaction between solute and the micellar phase. In other words, when $\Delta\Delta G$ values are more negative, partitioning of the solute with the micellar phase becomes more favorable. The first six solutes in Fig. 5 are NHB solutes. Although dipolarity and polarizability can influence their retention, the hydrophobic interaction plays the major role in electrokinetic retention of NHB solutes. The graphs of the n-octanol/water partition coefficient ($\log P_{ow}$) versus $\Delta\Delta G$ values of the 14 test solutes (Fig. 5) reveal high coefficients of determination, R^2, in all surfactant systems (graph not shown). The R^2 values for SUS, 80:20, 60:40, 50:50, 40:60, 20:80, SUL are 0.832, 0.907, 0.932, 0.941, 0.954, 0.941, and 0.970, respectively (R^2 for SDS is 0.868). With the exception of 20:80, a steady increase in R^2 value as a factor of SUL concentration is obvious. This observation indicates that an increase in hydrophobic character of the solute, e.g., an increase in the alkyl chain length of the functional group such as

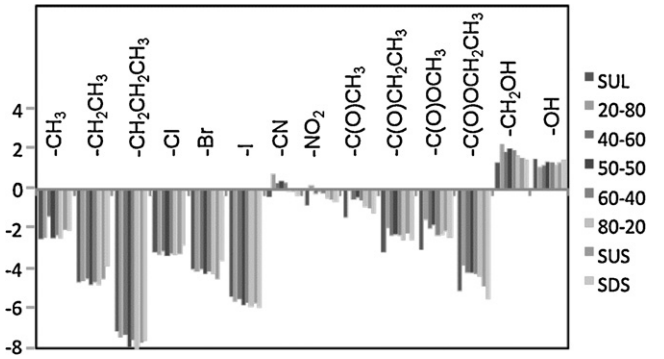

Fig. 5. The $\Delta\Delta G$ values for various functional groups as a factor of percent mole fraction of the surfactants. The legends are given on the graph.

$-CH_3$, $-C_2H_5$, and $-C_3H_7$, results in an increase in the strength of the interaction between alkylbenzene and the surfactant systems. For halogenated NHB solutes, the $\Delta\Delta G$ values decrease with increases in the size (thus hydrophobicity) of the solute. In general, the most negative $\Delta\Delta G$ values were obtained for NHB solutes in all pseudostationary phases studied. The HBA solutes in the list (except benzonitrile, nitrobenzene and acetophenone) have the second largest negative $\Delta\Delta G$ values due probably to their hydrophobic characters and hydrogen bond basicities. It should be noted that benzonitrile ($\log P_{ow} = 1.56$), nitrobenzene ($\log P_{ow} = 1.85$) and acetophenone ($\log P_{ow} = 1.58$) have smaller negative or positive $\Delta\Delta G$ values due to their low hydrophobic character. If the solute location inside the micelle is alkane-like, one would expect these benzene derivatives to significantly favor the aqueous buffer phase. The fact that they do not implies that the solute location inside the micelle is significantly hydrated. The least negative (or positive) $\Delta\Delta G$ values were obtained for the HBD solutes, benzyl alcohol ($\log P_{ow} = 1.08$) and phenol ($\log P_{ow} = 1.49$), which can be attributed to the hydrophilic properties and weakest influence of the *a* coefficient on electrokinetic retention.

3.5. The effect of surfactant composition on distribution coefficients and chemical selectivity

Selectivity differences between the pseudostationary phases can be compared by plotting $\log k$ values against each other [47]. If selectivity between all surfactants were the same, a linear plot with all points falling on the line with a value of *R* closer to 1.0 would be observed. Alternatively, a scatterplot would indicate selectivity difference between the surfactant systems. The comparison of $\log k$ values shows the similarities between the selectivity of SUS and SDS surfactant systems (Fig. 6A). However, slight difference is noticeable. For example, majority of the acidic HBD solutes fall under the trend line (of NHB solutes) indicating a slight tendency towards SDS, which is relatively more basic than SUS. The selectivity difference between SUS and SUL is more pronounced (Fig. 6B). All basic HBA solutes interact strongly with relatively hydrogen bond acidic SUS while acidic HBD prefer basic SUL surfactant. Another indication of selectivity difference is the correlation coefficient (R^2). The R^2 of the regression lines of $\log k$ for SUS versus the $\log k$ for the remaining surfactant systems were determined (data not shown). A gradual decrease in R^2 values was observed as the mole fraction of SUL was increased. The R^2 values between $\log k$ for SUS and 80:20, 60:40, 50:50, 40:60, 20:80, SUL and SDS were 0.968, 0.954, 0.946, 0.933, 0.926, 0.866, and 0.961, respectively. A low R^2 value indicates the selectivity difference between the two compared surfactant systems. Although the differences are not prominent, the data show that the selectivity can be manipulated by varying the surfactant concentration in a mixed surfactant system.

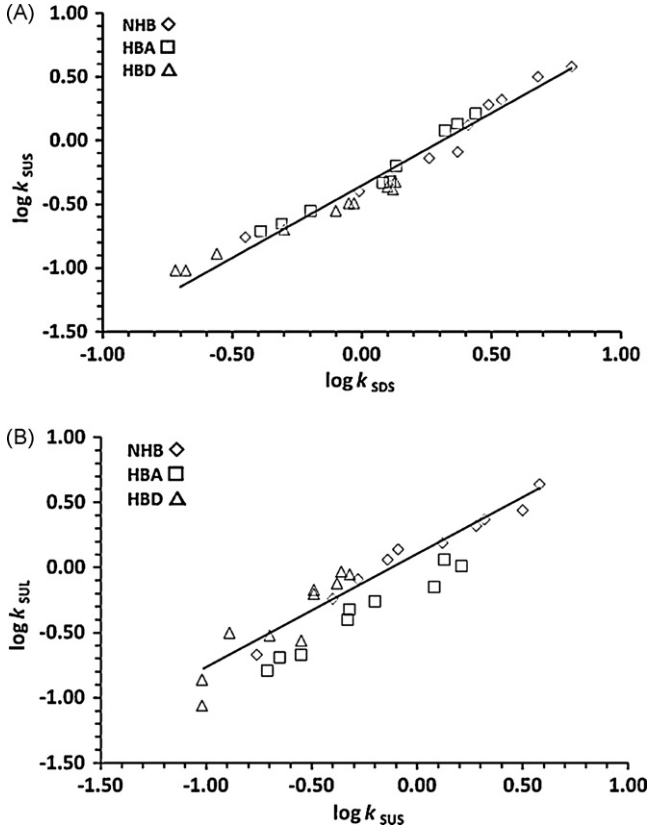

Fig. 6. Representative capacity factor comparison between (A) SDS and SUS, (B) SUS and SUL. Legends are shown in the plot. The solid line represents the trend line for the NHB solutes.

4. Concluding remarks

The binary mixtures of monomeric SUS, an achiral surfactant with sulfate head group, and monomeric SUL, a chiral surfactant with leucinate head group, were characterized using a variety of analytical chemistry techniques. The CMC values of SUS and SUL are the highest and the lowest, respectively, and those of conventional mixed micelles are very close to that of SUL monomer. The C_{20} values decreased dramatically with an increase in the concentration of SUL in the mixed micelle. The SDS and SUS showed the highest and the lowest surface activity, respectively, as supported by their lowest and the highest γ_{CMC} values; however, the surface activities of SUL and the binary mixed surfactants were found to be very similar. The SUS had the lowest partial specific volume suggesting that the hydration of its micelles was small which resulted in a relatively more compact structure. Due probably to the longer carbon chain in its hydrophobic tail and larger hydration of its micelles, SDS had relatively larger partial specific volume than SUS. The partial specific volume of SUL is largest among three single surfactants due probably to its bulky leucinate head group. A further increase in the content of SUL to 80% resulted in a more flexible mixed surfactant with larger hydration. Among the eight pseudostationary phases tested, SDS provided the most hydrophobic environment (highest methylene group selectivity). Due to the presence of hydrophobic leucinate head group, SUL was second most hydrophobic whereas SUS was among the least hydrophobic surfactants. An increase in SUL mole percentage gradually increased the α_{CH_2} values making the mixed surfactants more hydrophobic. A steady increase in μ_{eo} values was observed as the percent mole fraction of SUL was increased from 20 to 80. It was noted that SDS had the highest; whereas SUL and SUS had the lowest absolute μ_{ep} values. The rest of the pseudostationary phases, however, had approximately the same μ_{ep} values. The migration-time window was found to be the widest for SUS, while all other surfactant systems had very similar values. Under experimental conditions used, SDS had the lowest and SUL had the highest phase ratio among all surfactant systems studied. A correlation was found between the migration-time window and the γ_{CMC} values. Correlation among phase ratio, γ_{CMC} of the surfactant systems and their electrophoretic mobilities in MEKC was also visible with the exception of SUL system.

The LSER was applied for characterization of the retention and selectivity of pseudostationary phases in MEKC. The cohesiveness and the hydrogen bond acidic character of the surfactant systems were found to have the most significant influence on selectivity and MEKC retention. The SUS and SDS showed the strongest while SUL showed the weakest hydrogen bond donating capacity.

The basicity, interaction with n and π-electrons of the solute and dipolarity/polarizability were the least significant factors in LSER model for the surfactant systems studied. Free energies of transfer of selected functional groups in each surfactant systems were also calculated and found to be in good agreement with the LSER data.

Acknowledgments

This work was supported by a Support of Competitive Research (SCORE) Program grant from the National Institute of General Medical Sciences, one of the National Institutes of Health (grant no. S06GM078246). David Ahlstrom acknowledges the financial support of the Fayetteville State University Research Initiative for Scientific Enhancement (FSU-RISE) Program. Yatzka Hoyos is thankful for the financial support from North Carolina Louis Stokes Alliance for Minority Participation (NC-LSAMP) Program at Fayetteville State University.

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] D.R. Baker, Capillary Electrophoresis, Wiley-Interscience, New York, 1995, p. 55.
[3] E. Fuguet, C. Ràfols, E. Bosch, M.H. Abraham, M. Rosés, Electrophoresis 27 (2006) 1900.
[4] C.P. Palmer, J. Sep. Sci. 31 (2008) 783.
[5] E. Fuguet, C. Ràfols, E. Bosch, M.H. Abraham, M. Rosés, J. Chromatogr. A 942 (2002) 237.
[6] K.R. Nielsen, J.P. Foley, J. Microcol. Sep. 5 (1993) 347.
[7] M.D. Trone, J.P. Mack, H.P. Goodell, M.G. Khaledi, J. Chromatogr. A 888 (2000) 229.
[8] M.D. Trone, M.S. Leonard, M.G. Khaledi, Anal. Chem. 72 (2000) 1228.
[9] R. Pascoe, J.P. Foley, Electrophoresis 23 (2002) 1618.
[10] C.L. Copper, M.J. Sepaniak, Anal. Chem. 66 (1994) 147.
[11] C. Akbay, S.A. Shamsi, I.M. Warner, J. Chromatogr. A 910 (2001) 147.
[12] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, Electrophoresis 15 (1994) 1273.
[13] C.F. Poole, S.K. Poole, M.H. Abraham, J. Chromatogr. A 798 (1998) 207.
[14] N. Chen, Y. Zhang, S. Terabe, T. Nakagawa, J. Chromatogr. A 678 (1994) 327.
[15] S. Yang, M.G. Khaledi, Anal. Chem. 67 (1995) 499.
[16] M. Rosés, C. Ràfols, E. Bosch, A.M. Martínez, M.H. Abraham, J. Chromatogr. A 845 (1999) 217.
[17] W. Shi, D.S. Peterson, C.P. Palmer, J. Chromatogr. A 924 (2001) 123.
[18] S. Schulte, C.P. Palmer, Electrophoresis 24 (2003) 978.
[19] M.J. Kamlet, R.W. Taft, J. Am. Chem. Soc. 98 (1976) 2886.
[20] M.J. Kamlet, R.M. Doherty, M.H. Abraham, Y. Marcus, R.W. Taft, J. Phys. Chem. 92 (1988) 5244.
[21] A.J. Platts, M.C. Du, M.H. Abraham, J. Org. Chem. 65 (2000) 7114.
[22] M.G. Khaledi, J.G. Bumgarner, M. Hadjmohammadi, J. Chromatogr. A 802 (1998) 35.
[23] J.P. McCarney, R.D. Loflin, E. Rauk, S. Yusa, C.P. Palmer, Electrophoresis 26 (2005) 841.
[24] S. Yusa, A. Sakakibara, T. Yamamoto, Y. Morishima, Macromolecules 35 (2002) 5243.
[25] S.A. Shamsi, C. Akbay, I.M. Warner, Anal. Chem. 70 (1998) 3078.
[26] J. Wang, I.M. Warner, Anal. Chem. 66 (1994) 3773.
[27] M. Lüscher-Mattli, in: H.-J. Hinz (Ed.), Thermodynamic Data for Biochemistry and Biotechnology, Springer-Verlag, New York, 1986, p. 276.
[28] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[29] J.P. Foley, Anal. Chem. 62 (1990) 1302.
[30] M.F. Vitha, P.W. Carr, Sep. Sci. Technol. 33 (1998) 2075.
[31] J.K. Armstrong, J. Parsonage, B. Chowdhry, S. Leharne, J. Mitchell, A. Beezer, K. Lohmer, P.J. Laggmer, Phys. Chem. 97 (1993) 3904.
[32] K.S. Sharma, S.R. Patil, A.K. Rakshit, K. Glenn, M. Doiron, R.M. Palepu, P.A. Hassan, J. Phys. Chem. B 108 (2004) 12804.
[33] J.A. Molina-Bolívar, J. Aguiar, C.C. Ruiz, J. Phys. Chem. B 106 (2002) 870.
[34] C.C. Ruiz, J.A. Molina-Bolivar, J. Aguiar, G. MacIsaac, S. Moroze, R. Palepu, Colloid Polym. Sci. 281 (2003) 531.
[35] A. Gavenda, P. Bednar, P. Bartak, P. Adamovsky, J. Sevck, P. Tzoumas, J. Ulrichova, J. Sep. Sci. 24 (2001) 723.
[36] M.H. Abraham, H.S. Chadha, G.S. Whiting, R.C. Mitchell, J. Pharm. Sci. 83 (1994) 1085.
[37] M.V. Vitha, P.W. Carr, J. Chromatogr. A 1126 (2006) 143.
[38] C. Fu, M.G. Khaledi, J. Chromatogr. A 1216 (2009) 1891.
[39] C.F. Poole, S.K. Poole, J. Chromatogr. A 792 (1997) 89.
[40] O.A. El Seoud, J. Mol. Liquids 72 (1997) 85.
[41] P. Mukarjee, J.S. Ko, J. Phys. Chem. 96 (1992) 6090.
[42] D. Evans, B.W. Ninham, J. Phys. Chem. 87 (1983) 5025.
[43] M.F. Ottaviani, P. Baglioni, G. Martini, J. Phys. Chem. 87 (1983) 3146.
[44] S. Ghosh, M. Petrin, A.H. Maki, J. Phys. Chem. 90 (1986) 5206.
[45] M.F. Borgerding, R.L. Williams, W.L. Hinze, F.H. Quina, J. Liquid Chromatogr. 12 (1989) 1367.
[46] A.A. Agbodjan, M.G. Khaledi, J. Chromatogr. A 1004 (2003) 145.
[47] W. Melandera, J. Stovekena, C. Horvátha, J. Chromatogr. 199 (1980) 35.