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Characterization and application of molecular binary mixed molecular micelles of sodium 10-undecenyl sulfate and sodium *N*-undecenyl leucinate as pseudostationary phases in micellar electrokinetic chromatography

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ABSTRACT

Poly (sodium 10-undecenyl sulfate) (poly SUS), poly (sodium 10-undecenyl leucinate) (poly SUL), and their five molecular binary mixed micelles with varied SUS:SUL composition were prepared. The purity of these molecular micelles was confirmed by elemental analysis. Their partial specific volume, aggregation number, methylene selectivity, polarity, phase ratio, mobility, and elution window values were determined using a variety of analytical techniques. These molecular micelles were then evaluated as pseudostationary phases in micellar electrokinetic chromatography (MEKC) for separation of benzene derivatives with a wide range of chemical properties. Elemental analysis results reveal that the ratio of the two surfactants in the binary mixture does not change significantly during the polymerization process. Poly SUS was found to have the lowest partial specific volume and it increases gradually with an increase of SUL mole fraction. Poly SUL was found to provide the most hydrophobic environment for test solutes. Based on the retention results, the strength of interaction between the molecular micelles and the analytes was found to follow the following order: NHB > HBA > HBD. This order indicates that the hydrophobic interaction plays a major role in retention of benzene derivatives.

1. Introduction

Micellar electrokinetic chromatography (MEKC) extends the application range of capillary electrophoresis by providing a mechanism for separation of neutral as well as ionic solutes [1,2]. The separation in MEKC is based on the partitioning of analytes between the mobile phase (buffer solution) and pseudostationary phase (micellar phase). A number of new pseudostationary phases with diverse selectivities have been introduced to perform separation of different sets of chemicals [3–7]. Although successfully used as pseudostationary phases in many separation applications, conventional micelles have some drawbacks as pseudostationary phases in MEKC [3–8]. Molecular micelles (or polymeric micelles) have been introduced as supplemental or alternative pseudostationary phases to conventional micelles in MEKC [9–16]. They provide several advantages over conventional micelles [7,12]: (a) they have zero CMC, that is, they may be used at low concentrations below the normal CMC of the monomer; (b) they are stable in the presence of high content of organic modifiers; this is because monomers are covalently bonded together and organic additives do not disrupt the primary covalent structure of the micelle polymer; (c) due to their high molecular weight, molecular micelles can conveniently be used in MEKC–mass spectrometry applications without background interference from low-molecular-weight monomers.

Amino-acid based surfactants have received significant amount of attention due to their low toxicity, biocompatibility and fast biodegradation, their effectiveness against certain bacteria, viruses and tumors [17] and their application in separation science [9–16,18–20]. Previously in our laboratory, sodium 10-undecenyl sulfate (SUS), a sulfate-based achiral surfactant, and sodium *N*-undecanoyl L-leucinate (SUL), an amino acid-based chiral surfactant, were synthesized, polymerized at certain percent mole fractions to form molecular mixed-micelles and were subsequently applied as novel pseudostationary phases in MEKC for separation of chiral and achiral benzodiazepines [18–20]. Modification of the chemical composition by varying the percent ratios of SUS and SUL in their binary mixtures resulted in novel pseudostationary phases with different selectivities towards benzodiazepines [18].

There are several objectives for the current study. First, we intend to manipulate the selectivity of pseudostationary phases by varying the percent ratios of the two surfactants in their binary mixtures. Second, we seek to increase the solubility of amino acid-based surfactants at acidic conditions, since they precipitate out of solution

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below pH 7.0 due to the presence of carboxylate head groups. The solubility of SUL can be increased in acidic pHs by mixing it with a highly soluble sulfated surfactant (e.g., SUS). Although SUL surfactant precipitates at acidic pHs (e.g., below pH 7.0) due to the protonation of carboxylate head group, SUS micelles are believed to solubilize and keep SUL monomers in the micelles. Third, the binary molecular mixed-micelles with both carboxylate and sulfate head groups can be used as pH-responsive pseudostationary phases. The charge density and possibly the conformation of the molecular mixed-micelle of SUS and SUL vary at acidic and basic conditions due to the protonation of caboxylate moiety. This may affect the performance and selectivity of the mixed micelles. A pH-responsive polymer with sulfonate and carboxylate head groups, poly(sodium 2-(acrylamido)-2-methylpropanesulfonate/11-(acrylamido)-undecanoic acid))(poly(NaAMPS/AmU) was introduced recently [21]. At low pHs, the sulfonic acid groups remain ionic whereas the carboxylate groups are neutral. 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 the carboxylate groups changes the balance between ionic repulsion and hydrophobic interaction. As a result of this alteration, poly(-NaAMPS/AmU) was found to form a compact conformation at acidic pHs and a more open configuration at basic pHs. The authors found that the change in conformation had significant effects on the electrophoretic mobility, retention, selectivity, and separation efficiency. Higher electrophoretic mobility and a greater affinity for majority of solutes were observed at lower pHs. In addition, highly hydrophobic solutes with long alkyl chains were found to migrate with a better efficiency at lower pHs.

In the present work, we attempt to further characterize molecular micelles of SUS and SUL as well as their five molecular binary mixed micelles. The composition of these novel molecular micelles was verified by elemental analysis. They were then applied as pseudostationary phases in MEKC for separation of benzene derivatives with varied chemical properties. Their partial specific volumes, aggregation numbers, methylene selectivity, polarity, mobility, and elution windows were also determined using a variety of analytical techniques.

2. Experimental

2.1. Materials

All benzene derivatives, alkyl phenyl ketone homologs, *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) and a water purification system from Millipore (Milford, MA, USA), respectively. All chemicals were used in the received form without further purification.

2.1.1. Synthesis of SUS and SUL and their polymerizations

Details of the synthesis of SUS [12] and SUL [22] as well as their polymerization and copolymerization [23] are available in the literature, thus are not repeated in this report. The chemical structures of SUS and SUL are given in Fig. 1. The representative micellar structure of SUS–SUL binary mixture with 50:50 mol fraction and the molecular micelle of the same binary mixture after polymerization by gamma radiation is also represented in Fig. 1.

2.1.2. Preparation of separation buffers and solute solutions

A 1.0 M solution of each of anhydrous NaH_2PO_4 and Na_2HPO_4 was prepared by dissolving appropriate amounts of each compound in deionized water. After 10-fold dilution, a mixture of NaH_2PO_4 solution (42.3 mL) and Na_2HPO_4 solution (57.7 mL) provided a stock solution of 100 mM phosphate buffer with a pH of 7.0. Appropriate amount of molecular micelle was added to a given volume of buffer solution to produce 1.0% (v/w) surfactant concentration and pH was adjusted to 7.00 using either NaOH or HCl, if necessary, and the final volume was adjusted with deionized water. The final concentration of phosphate buffer in all background electrolytes was adjusted to 10.0 mM. Each run buffer was sonicated for 2 min, filtered through a 0.45 μm syringe filter (Nalgene, Rochester, NY, USA), and then degassed for one additional minute before use in MEKC experiments. All stock test solute solutions were prepared in methanol with a concentration of 20 mg/mL each and were diluted about 10 fold before injection.

2.2. Characterization of surfactants

2.2.1. Elemental analysis

Percent molar ratios of SUS and SUL in the molecular micelles were confirmed by elemental analysis. Elemental analysis experiments were conducted by researchers at the University of Texas at Arlington.

2.2.2. Determination of CMC and partial specific volume

Since the surfactant monomers are covalently linked to each other in micellar form, the CMC of molecular micelles is assumed to be zero. Partial specific volume, $\bar{v}$, is defined as an increase in the volume upon dissolving 1.0 g of a dry surfactant material in a large volume of a solvent at constant temperature and pressure. Since the measurement of such small volume change is nearly impossible, an approach based on density measurement of the

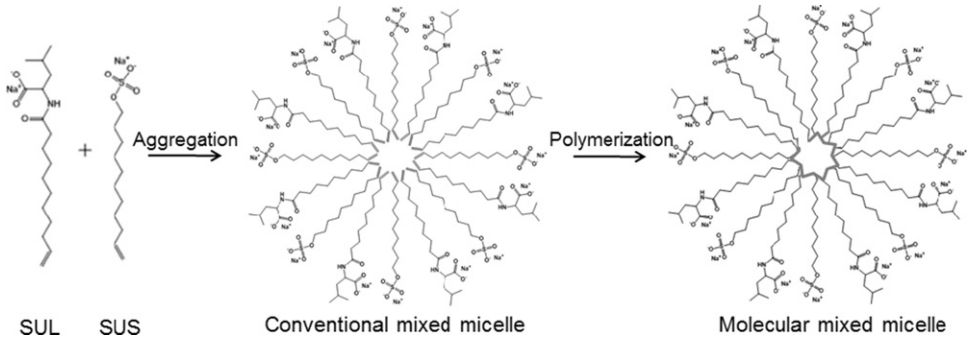

Fig. 1. Chemical structures of SUS and SUL as well as representative chemical structure of SUS–SUL binary mixture with 50:50 mol% fraction and molecular micelle after polymarization by gamma radiation.

surfactant solutions has been used for determination of the partial specific volume values with high precision [24,25]. A graph of $1/\rho$ against weight fraction of solvent (W) allows the determination of partial specific volume from the y-intercept value. The solutions for density measurements were prepared using the following procedure: A 0.10 g/mL stock solution of each of poly SUS, Poly SUL and the five molecular binary mixed micelles was prepared in deionized water. About 3 mL of five different concentrations with 0.02 g/mL increments (including the stock solution) was prepared from the stock solutions. To determine the weight fraction of the solvent, individual solutions were weighed using a sensitive digital scale and the mass of surfactant in each solution was calculated before the density measurements were carried out. Finally, the densities of the prepared solutions were measured at 25 °C using a high-precision digital DMA 4500 density meter (Anton Paar, Ashland, VA, USA).

2.2.3. Determination of aggregation number and polarity

Aggregation number of the molecular micelles was determined by using the fluorescence quenching method proposed by Turro and Yekta [26]. Fluorescence measurements were performed at ambient temperature using a Shimadzu RF-5301PC spectrofluorophotometer. The Panorama Fluorescence software (version 1.1) was used for system control and data analysis. Pyrene and cetylpyridinium chloride were used as fluorescent probe and quencher, respectively. Fluorescence spectra of pyrene were recorded at several quencher concentrations. An increase in CPyCl concentration decreases the fluorescence intensity of pyrene molecule in micellar solution. The aggregation number, N, of each micelle was obtained from the slope of the ln (I0/IQ) versus [Q] plot. Experimental section and calculation for determination of aggregation number are detailed elsewhere [16], thus were not repeated here. Polarity of surfactant systems was also estimated by the fluorescence method. Pyrene solution shows five vibrational bands in fluorescence spectra. Ratio of the first band at 373 nm (I_1) and the third band at 384 nm (I_3) shows a strong dependence on hydrophobicity of pyrene microenvironment [27], thus, I_1/I_3 value in each of the molecular surfactant system was taken as its polarity.

2.3. Capillary electrophoretic separations

2.3.1. 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 the 3D-CE ChemStation (Rev. B.03.01) 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.

2.3.2. 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 2 min with triply deionized water and for 2 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). All MEKC separations were performed at a constant voltage of +30 kV, and the capillary temperature was fixed at 25 °C. Unless otherwise noted, the injection size was 50 mbar. Peaks were identified by the comparison of their individual UV-spectra obtained from diode array detector or via spiking when necessary.

2.4. Calculations

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

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

where t_R, t_{eof} and t_{psp} are the migration times of solute, EOF, and the pseudostationary phase, respectively. Methanol and undecanophenone were used to measure t_{eof} and t_{psp} markers, respectively. The apparent electrophoretic mobility of pseudostationary phase, μ_{app}, was calculated at 25 °C by using Eq. (2):

$$\mu_{app} = \frac{I_t I_d}{V t_{psp}} \quad (2)$$

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. The migration times were measured in seconds. The effective electrophoretic mobility of pseudostationary phases (μ_{ep}) was calculated from the difference between μ_{app} and electroosmotic mobility, μ_{eo}, (i.e., $\mu_{ep} = \mu_{app} - \mu_{eo}$). To calculate the μ_{eo} of the buffer solution, t_{psp} term in Eq. (2) was replaced with t_{eof}.

The phase ratio of the surfactant system, β, was determined using Eq. (3) [28,29]:

$$\beta = \frac{V_{psp}}{V_{aq}} = \frac{\overline{\nu}([S_{tot}] - \mathrm{CMC})}{1 - \overline{\nu}([S_{tot}] - \mathrm{CMC})} \quad (3)$$

where V_{psp} is the volume of pseudostationary phase (micellar phase) and V_{aq} is the volume of the aqueous mobile phase. The $\overline{\nu}$, $[S_{tot}]$, and CMC are partial specific volume, total concentration, and CMC of the pseudostationary phase, respectively. Since the CMC values for molecular micelles are considered to be zero, Eq. (3) can be simplified as

$$\beta = \frac{\overline{\nu}[S_{tot}]}{1 - \overline{\nu}[S_{tot}]} \quad (4)$$

The distribution coefficient, K, is related to k by the following equation [1,30]:

$$K = \frac{k}{\overline{\nu}([S_{tot}] - CMC)} \quad (5)$$

Elution window was calculated by use of t_{psp}/t_{eof} ratio and methylene selectivity, α_{CH_2}, was calculated from the antilogarithm of the slope of the regression line of log k versus carbon number of alkyl phenyl ketone homologous series.

3. Results and discussion

The primary structural difference between the pseudostationary phases SUS and SUL is the head groups attached to the C11 backbone. The sulfate and the leucinate are the charge-bearing regions for SUS and SUL, respectively, whereas sodium is a counter ion associated with the C11 hydrophobic tail. In this study, the effect of varied percent mole fractions of these two surfactants in their molecular micelles will be examined and the selectivity differences of these pseudostationary phases will be compared.

3.1. Elemental analysis

Whether or not the ratio of SUS and SUL in binary mixed micelles is preserved during the polymerization process is one of the major concerns in application of molecular micelles as pseudostationary phases in MEKC. Since each surfactant has a distinctive element (i.e., sulfur in SUS and nitrogen in SUL), elemental analysis can be practically used for structural confirmation. The weight percentage of nitrogen (or sulfur) can be related to the content of SUL (or SUS) in the molecular binary mixed micelles. As seen in Table 1, the weight percentage of nitrogen gradually increases, while that of sulfur decreases, with an increase in the content of SUL. The elemental analysis data are fairly close to the theoretical values suggesting that the ratio of the two surfactants in the binary mixed micelles does not change significantly during the polymerization process. Slight differences between the theoretical and experimental values are, probably, due to the presence of a small amount of water associated with the polymers.

3.2. Effect of molecular mixed-micelle composition on partial specific volume and phase ratio

Partial specific volumes and phase ratio values of the molecular micelles are listed in Table 2. Partial specific volume is closely related to the hydration of micelle [31,32]; therefore, an increase in partial specific volume can be attributed to the number of water molecules in the Stern and palisade layers of the micelle. Conversely, a decrease in partial specific volume may be due to the dehydration of the micelle, which results in a relatively more compact micellar structure [33,34]. As can be seen in Table 2, the partial specific volume of poly SUL (0.820 cm^3/g) is greater than that of poly SUS (0.755 cm^3/g), indicating that poly SUL, which has more carbon atoms in its leucinate head group, holds more water molecules in its Stern and palisade layers as compared with poly SUS. All mixed molecular micelles have partial specific volume values between that of poly SUS and poly SUL. There is a noticeable steady increase in partial specific volume as a factor of percent mol fraction of SUL in the micelle. For example, partial specific volume increased from 0.762 cm^3/g to 0.802 cm^3/g with an increase in mole fraction of SUL from 20% [poly (80:20)] to 80% [poly (20:80)]. It is worth mentioning that the partial specific volumes of monomeric SUS (0.800 cm^3/g) and SUL (0.874 cm^3/g) are greater than those of corresponding molecular micelles of SUS and SUL [35]. This may indicate the fact that the monomeric micelles are relatively more hydrated than their polymers. The rigidity of the molecular micelles maybe due to the covalent bonds between the surfactant monomers in the molecular micelles, which results in relatively more compact aggregates.

The phase ratio values, β, of the surfactant systems are also listed in Table 2. Under MEKC conditions studied (e.g., lower pseudostationary phase concentration relative to the aqueous phase), the volume of the micellar phase is expected to be much smaller than the aqueous phase, thus, V_{psp}/V_{aq} is expected to be lower. Poly SUS system provided the largest phase ratio (0.0285) among the pseudostationary phases studied. The phase ratio of poly SUL system is 0.0268, which is smaller than that of poly SUS

Table 1
Elemental analysis of the molecular micelles.

		Carbon%		Hydrogen%		Nitrogen%		Sulfur%	
		Theoretical	Experimental	Theoretical	Experimental	Theoretical	Experimental	Theoretical	Experimental
Surfactant systems	Poly SUS	48.51	47.56 (±0.11)[a]	7.78	8.27 (±0.09)	0.00	0.12 (±0.06)	11.75	10.13 (±1.40)
	Poly (80:20)	52.36	49.51 (±0.03)	8.16	8.54 (±0.03)	0.99	0.98 (±0.04)	9.01	8.75 (±0.50)
	Poly (60:40)	55.87	52.82 (±0.19)	8.57	8.58 (±0.11)	1.89	1.95 (±0.04)	6.48	5.86 (±0.06)
	Poly (50:50)	57.52	54.11 (±0.04)	8.76	9.11 (±0.06)	2.31	2.25 (±0.06)	5.25	3.85 (±1.79)
	Poly (40:60)	59.11	55.66 (±0.39)	8.94	9.51 (±0.13)	2.72	2.60 (±0.03)	4.15	3.49 (±0.64)
	Poly (20:80)	62.09	57.87 (±0.06)	9.29	9.58 (±0.11)	3.49	3.37 (±0.04)	2.00	2.17 (±0.08)
	Poly SUL	64.82	59.67 (±0.04)	9.68	10.10 (±0.13)	4.20	3.99 (±0.05)	0.00	1.27 (±0.04)

[a] Each surfactant was analyzed at least twice.

Table 2
Physicochemical properties of the investigated molecular micelles.

Physicochemical property	Pseudostationary phase						
	Poly SUS	Poly (80:20)	Poly (60:40)	Poly (50:50)	Poly (40:60)	Poly (20:80)	Poly SUL
CMC[a] (mM)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Partial specific volume[b], $\bar{v}$(mL g^{-1})	0.755	0.762	0.782	0.785	0.792	0.802	0.820
Phase ratio[c], β	0.0285	0.0278	0.0276	0.0273	0.0271	0.0266	0.0264
Effective electrophoretic mobility[d, e], μ_{ep} (10^{-4} cm^2 V^{-1} s^{-1})	−4.75	−4.65	−4.64	−4.69	−4.53	−4.41	−4.20
Methylene-group selectivity[d, f], α_{CH_2}	2.33	2.20	2.19	2.14	2.18	2.18	2.67
Aggregation number[g]	97	88	102	84	77	53	61
Polarity[g]	0.84	1.24	1.24	1.24	1.18	1.15	1.11
Migration-time window[d], t_{psp}/t_{eof}	3.9	3.5	3.2	3.1	3.4	2.8	2.9

[a] Critical micelle concentration of molecular micelles is assumed to be zero.
[b] Determined in deionized water at 25 °C by density meter.
[c] Eq. (4) was used for phase ratio determinations.
[d] Data were collected with 66 cm (57.5 cm effective length) × 50 μm ID capillary with an applied voltage of +30 kV using 10 mM phosphate buffer at pH of 7.0; temperature, 25 °C.
[e] μ_{ep} was calculated using $\mu_{ep}=\mu_{app}-\mu_{eo}$.
[f] 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)].
[g] Determined using the fluorescence quenching method at ambient temperature (~25 °C).

but very similar to the phase ratio of poly 20:80 (0.0266). As seen in Table 2, a steady decrease in phase ratio is observed as a function of increased SUL content. It should be noted that the phase ratios of the molecular mixed-micelles are lower than their parent monomeric mixed micelles [35]. It is interesting to note that the molecular micelle with highest phase ratio (poly SUS, 0.0285) has the highest (least negative) constant *c* value (−2.351) in the linear solvation energy relationship (LSER) model [23]. The relationship between coefficient *c* and phase ratio is an indication of the fact that the former contains the phase ratio when the capacity factor is used as the dependent variable. However, the coefficient *c* is not always well correlated with the phase ratio because it contains some other system offsets as well. This discrepancy can be seen in the case of poly SUL, which has the lowest phase ratio (0.0264) but has one of the highest c constants (−2.478). Coefficient *c* is also dependent on the size of the molecular micelle as well as the concentration of surfactant and micelle.

3.3. Effect of molecular mixed-micelle composition on methylene selectivity, polarity and aggregation number

Methylene selectivity, α_{CH_2}, polarity and aggregation number values are listed in Table 2. Poly SUL has higher α_{CH_2} value (2.67) than poly SUS (2.33) probably due to greater hydrophobic character of leucinate head group compared with sulfate head group. All α_{CH_2} values for molecular binary mixtures are practically the same and lower than those of poly SUS and poly SUL. Molecular micelles have lower α_{CH_2} values (i.e., are less hydrophobic) than their monomeric forms, except for poly SUL, which is more hydrophobic than its monomer SUL [35].

Pyrene solution shows five vibrational bands in fluorescence spectrum. Intensity ratio of the first band (I_1) at 373 nm and third band (I_3) at 384 nm shows strong dependence on hydrophobicity of microenvironment. For example, I_1/I_3 value is around 0.6 and 1.8 in hydrocarbon solvents and water, respectively [27,36]. Polarity values listed in Table 2 show that pyrene senses the most hydrophobic microenvironment in poly SUS among the molecular micelles studied. Poly SUL and poly 80:20 molecular micelles are slightly more polar than poly SUS. The rest of the surfactant systems show very similar polar properties and are relatively more polar than poly SUS and poly SUL.

The aggregation number, *N*, values for poly SUS ($N=97$) and poly (60:40) ($N=102$) are very similar and highest among the surfactant systems studied. On the contrary, poly (20:80) ($N=53$) and poly SUL ($N=61$) have the lowest *N* values. The N values of the rest of the molecular micelles range from 77.to 88. Evaluation of *N* values suggests that and there is no apparent correlation between the molar fraction of SUL and *N* values; however, no clear explanation is currently available to justify the *N* trend of these molecular micelles. It is worth mentioning that the *N* values reported in this study are significantly different, especially those of poly SUS ($N=97$ in this study versus $N=21$ and $N=48$ in previous two studies), from those reported earlier, except for

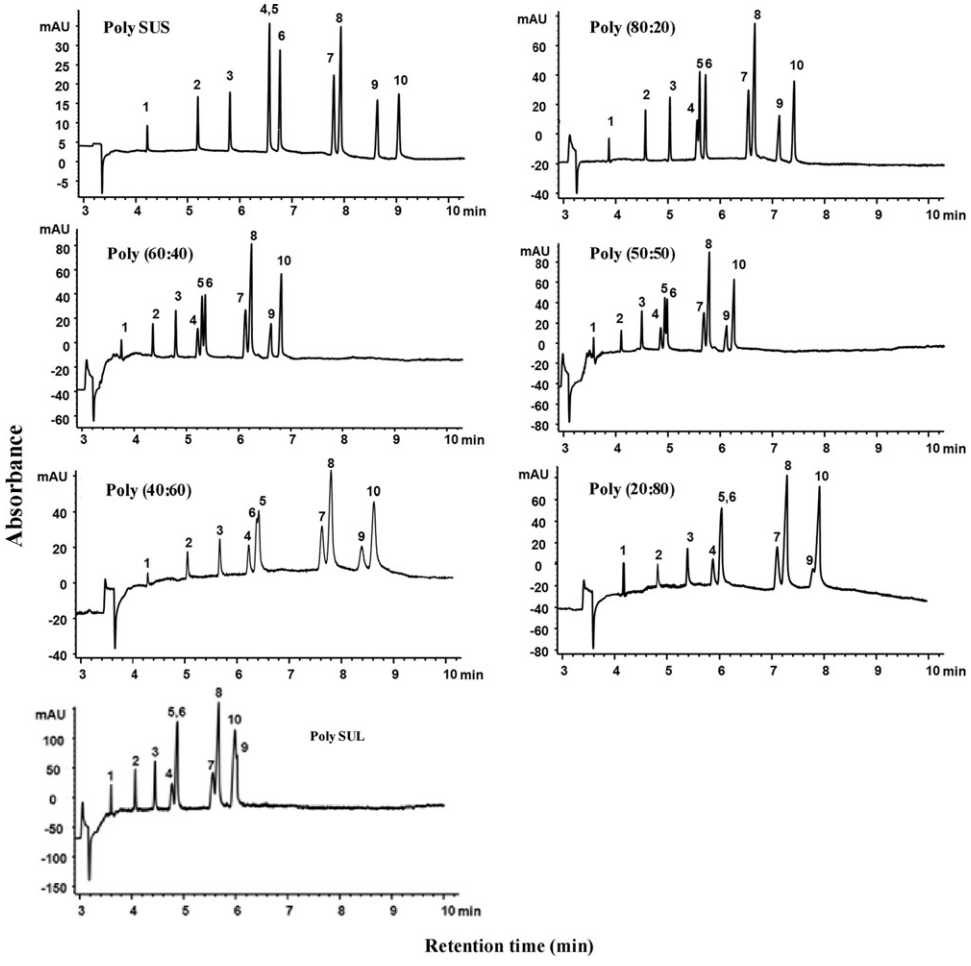

Fig. 2. Electropherograms showing the comparison of the seven molecular micelles as pseudostationary phases for separation of NHB benzene derivatives. MEKC separation conditions: 1.0% w/v each surfactant in 10 mM phosphate buffer (pH 7.0); pressure injection, 50 mbar for 1 s; applied voltage, +30 kV; temperature, 25 °C; UV detection, 200 nm. Peak identifications are same as listed in Table 3.

those of poly SUL, which are the same in both studies [19]. These discrepancies might be due to the polymerization processes taken place under different conditions (e.g., strength of gamma radiation source and length of the polymerization time). Length of the polymerization time (due to the strength of gamma radiation source) is believed to have a significant effect on the *N* values due to the fact that during the polymerization process the surfactant monomers in monomeric micelles may undergo different dynamic and/or kinetic processes that might generate diverse aggregates. For example, one-week-long polymerization time generated molecular micelles of poly SUS with an *N* value of 21, probably due to a weaker gamma radiation source, [19] whereas, 30-hours polymerization time that generated poly SUS molecular micelles with an *N* value of 48, due to a relatively stronger gamma source [16]. The gamma radiation source employed in the current study was stronger than that in the two previous studies and hence required shorter polymerization time and generated molecular micelles of poly SUS with an *N* value of 97.

3.4. Effect of molecular mixed-micelle composition on mobilities and migration-time window

The effective electrophoretic mobility (μ_{ep}) values were found to increase (become less negative) with an increase in SUL content, with an exception of poly (50:50), which has slightly higher mobility than poly SUS. The negative μ_{ep} value indicates that anionic pseudostationary phases are attracted to the anode in the opposite direction of electroosmotic movement. However, the stronger EOF drags the micelle polymers toward the cathode.

The migration-time window follows the reverse trend as compared with the electroosmotic mobility (Table 2). The widest and narrowest migration-time windows are obtained with poly SUS (3.9 min) and poly SUL (2.9 min); or poly (20:80), (2.8 min). The time window gets narrower with an increase in percent mole fraction of SUL in molecular mixed-micelles with the exception of poly (40:60), which provides relatively wider separation window (3.4 min). The difference in time window for the surfactant systems can also be seen from electropherograms of non-hydrogen bond solutes (NHB), hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) benzene derivatives (Figs. 2–4).

3.5. Effect of molecular mixed-micelle composition on electrophoretic separation

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, analytes may reside in several regions of the micelle. For example, hydrophobic analytes (e.g., aromatic hydrocarbons) with polarizable electrons reside near the polar head group, while hydrophobic alkanes are believed to penetrate into the hydrophobic micellar core. Solutes with amphiphilic character have special interaction with the micelle and align themselves with the

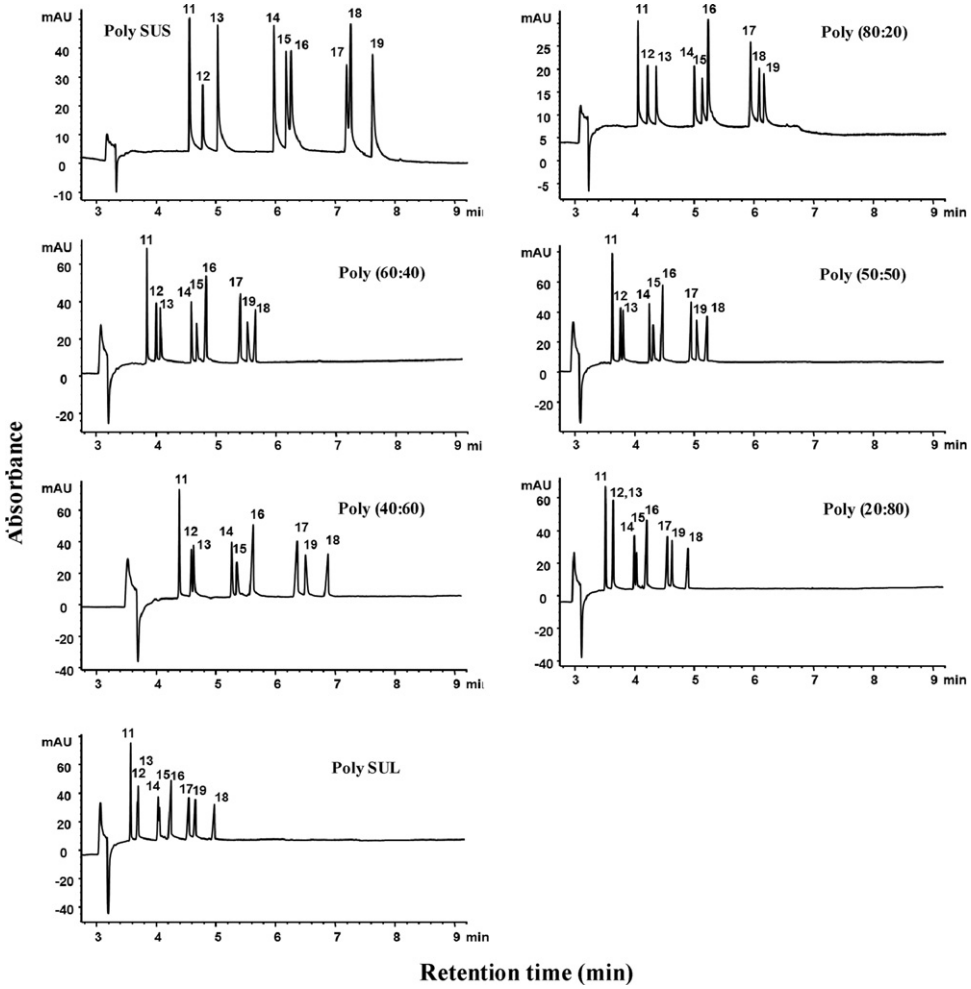

Fig. 3. Electropherograms showing the comparison of the seven molecular micelles as pseudostationary phases for separation of HBA benzene derivatives. MEKC separation conditions are same as in Fig. 1. Peak identifications are same as listed in Table 3.

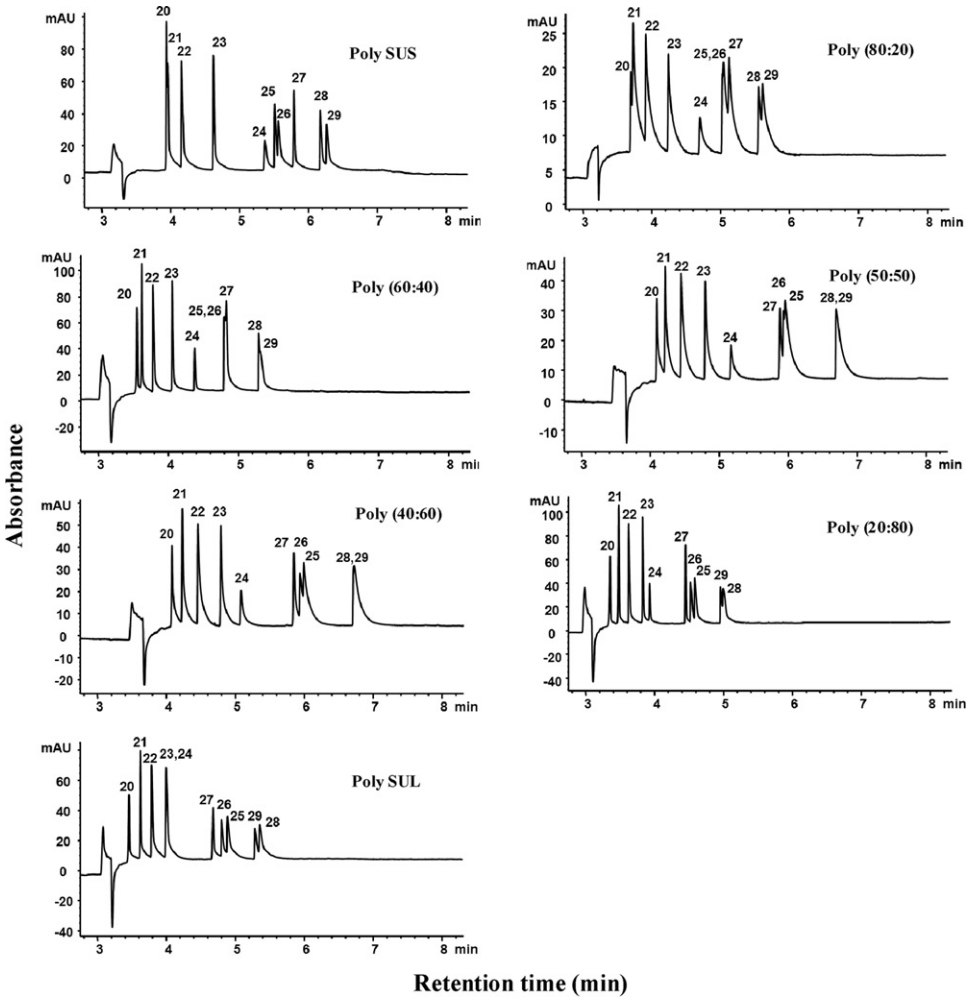

Fig. 4. Electropherograms showing the comparison of the seven molecular micelles as pseudostationary phases for separation of HBD benzene derivatives. MEKC separation conditions are same as in Fig. 1. Peak identifications are same as listed in Table 3.

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 analytes in each pseudostationary phase system is expected to be different.

To understand the mechanisms of solute interaction with the seven molecular micelles utilized in this work, retention behavior of 29 benzene derivatives with diverse properties was studied. The test benzene derivatives are informally classified as NHB, HBA, and HBD (Table 3). 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 HBA solutes possess only hydrogen bond accepting functional groups on the aromatic ring, whereas, the HBD solutes have both hydrogen bond donating and hydrogen bond accepting functional groups. Based on their pK_a values, all test solutes are considered to be neutral at pH 7.0.

Capacity factors, k, of the test solutes were calculated using Eq. (1); distribution coefficients, K, were then calculated from these k values by Eq. (5) and their logarithmic values, $\log K$, are listed in Table 3. The strength of interaction between pseudostationary phases and the solutes can be predicted from K values, since K is defined as the ratio of the concentration of solutes in micellar phase to the concentration in aqueous phase. Large $\log K$ values indicate that the solute retains longer in micellar phases than in aqueous phases. In general, the $\log K$ values for NHB solutes in molecular micelles increase with a decrease in the SUL content; however, poly (20:80) and poly (40:60) molecular mixed-micelles generate surprisingly high $\log K$ values. Similar trend is observed with HBA solutes, with the exception of poly (40:60) surfactant system. The HBD solutes show a different trend; the K values decrease slightly as the content of SUS is increased to 20% [poly (20:80)], then a sharp increase is observed up to 50% SUS content followed by a sharp decrease with poly (60:40) and a gradual increase in the remaining surfactant systems. Overall, $\log K$ values for solute subgroups can be given the order as NHB > HBA > HBD. Thus, NHB solutes retain the longest in all surfactant systems. Fastest and the slowest analysis times of all the three analyte groups were obtained with poly SUL and poly SUS, respectively. This retention behavior can be attributed to the major role of the hydrophobic interactions on the retention of solutes in MEKC.

As seen in Figs. 2–4, selectivity differences between pseudostationary phases toward the NHB solutes are apparent. For example, ethylbenzene (peak 4) and bromobenzene (peak 5) coelute in poly SUS but are resolved as the content of SUL in the molecular micelles is increased. An increase in SUL content, on the other hand, deteriorates the resolution between several adjacent compounds (e.g., bromobenzene/*p*-xylene, 4-chlorotoluene/iodobenzene and propylbenzene/naphthalene). Although not well resolved, the reversal in retention of propylbenzene (peak 9) and naphthalene (peak 10) with poly SUL is visible (Fig. 2).

Table 3
The list of NHB, HBA and HBD benzene derivatives as well as their distribution coefficients (log *K*).

Analytes		Pseudostationary phases						
		Poly SUS	Poly (80:20)	Poly (60:40)	Poly (50:50)	Poly (40:60)	Poly (20:80)	Poly SUL
NHB analytes								
1	Benzene	1.11	1.04	1.00	0.95	1.04	1.04	0.90
2	Toluene	1.51	1.40	1.36	1.32	1.43	1.45	1.26
3	Chlorobenzene	1.67	1.57	1.54	1.51	1.64	1.67	1.45
4	Ethylbenzene	1.83	1.72	1.67	1.62	1.81	1.84	1.58
5	Bromobenzene	1.83	1.73	1.70	1.65	1.85	1.90	1.61
6	p-Xylene	1.86	1.76	1.72	1.67	1.83	1.90	1.61
7	4-Chlorotoluene	2.08	1.94	1.93	1.88	2.17	2.26	1.83
8	Iodobenzene	2.10	1.99	1.96	1.90	2.21	2.33	1.86
9	Propylbenzene	2.24	2.09	2.05	2.00	2.38	2.55	1.96
10	Naphthalene	2.29	2.16	2.10	2.03	2.45	2.60	1.95
HBA analytes								
11	Benzonitrile	1.30	1.18	1.08	1.00	1.08	0.90	0.85
12	Nitrobenzene	1.38	1.26	1.18	1.11	1.18	1.04	1.00
13	Acetophenone	1.46	1.32	1.23	1.15	1.20	1.04	1.00
14	Methyl benzoate	1.72	1.56	1.46	1.40	1.48	1.30	1.26
15	Propiophenone	1.76	1.60	1.49	1.41	1.51	1.32	1.28
16	4-Nitrotoluene	1.78	1.63	1.54	1.49	1.60	1.40	1.38
17	4-Chloroacetophenone	1.97	1.82	1.73	1.66	1.80	1.56	1.52
18	4-Chloroanisole	1.99	1.86	1.81	1.75	1.93	1.69	1.68
19	Ethyl benzoate	2.05	1.87	1.77	1.69	1.83	1.59	1.56
HBD analytes								
20	Benzyl alcohol	1.00	0.90	0.78	0.90	0.78	0.70	0.70
21	Phenol	1.00	0.90	0.90	1.00	1.00	0.85	0.90
22	3-Methylphenol	1.11	1.08	1.04	1.18	1.11	1.00	1.08
23	4-Flourophenol	1.34	1.26	1.23	1.36	1.30	1.18	1.23
24	4-Chloroaniline	1.57	1.45	1.38	1.53	1.41	1.26	1.23
25	3-Chlorophenol	1.61	1.57	1.54	1.79	1.71	1.57	1.63
26	4-Chlorophenol	1.62	1.57	1.54	1.79	1.69	1.54	1.60
27	4-Ethylphenol	1.68	1.59	1.56	1.77	1.66	1.52	1.56
28	3-Bromophenol	1.76	1.72	1.71	2.02	1.89	1.72	1.79
29	4-Bromophenol	1.79	1.72	1.72	2.02	1.89	1.71	1.77

Similar selectivity differences are observed in adjacent HBA solutes (Fig. 3). The major difference is the change of the elution order of 4-chloroanisole (peak 18) and ethyl benzoate (peak 19). The later elutes as the last solute in poly SUS and poly (80:20) surfactant systems; the elution order is reversed as the content of SUL is increased. As seen in Fig. 3, adjacent propiophenone (peak 15) and 4-nitrotoluene (peak 16) as well as 4-chloroacetophenone (peak 17) and 4-chloroanisole (peak 18) peak pairs are partially resolved with poly SUS; however, both pairs are baseline resolved as the content of SUL in molecular micelle is increased. In contrast, an adverse trend is observed in adjacent nitrobenzene (peak 12) and acetophenone (peak 13) as well as methyl benzoate (peak 14) and propiophenone (peak 15); deterioration in resolution between these pairs occurs with an increase in SUL content.

All molecular micelles have substantial selectivity differences toward the HBD solutes (Fig. 4). For example, an apparent reversal in retention order of three adjacent pairs, i.e., phenol (peak 21)/benzyl alcohol (peak 20), 3-chlorophenol (peak 25)/4-chlorophenol (peak 26), 4-chlorophenol (peak 26)/4-ethylphenol (peak 27), and 3-bromophenol (peak 28)/4-bromophenol (peak 29), is observed. Notice that the major shift in retention order as a factor of SUL content in molecular micelle is observed in the last five solutes (peaks 25–29). One of the most prominent selectivity differences is the deterioration in resolution between 4-flourophenol (peak 23) and 4-chloroaniline (peak 24). These two solutes are well resolved with poly SUS, however, coelute with poly SUL.

The selectivity data signify that each of the co-polymerized molecular micelles can be used for separation of certain solutes with different physicochemical properties. In this preliminary data, it is apparent that some solutes, especially HBDs, exhibit strong tailing depending on the type of molecular micelle used. The tailing could be caused by the adsorption of solutes on the capillary wall or varying solute migration rates between the bulk buffer solution and pseudostationary phase. Using additives such as urea or lowering solute concentration can reduce tailing and hence improve the peak shape.

4. Conclusion

In conclusion, the elemental analysis data suggest that the ratio of SUS and SUL surfactant monomers in the binary mixed micelles does not change significantly during polymerization process. Molecular micelle with leucinate head group, poly SUL, is more hydrated than molecular micelle with sulfate head group, poly SUS, as suggested by their partial specific volume values. Based on the methylene selectivity values, poly SUL shows the highest hydrophobic character probably due to the relatively hydrophobic leucinate head group. Polarity values, on the other hand, indicate that pyrene senses the most apolar microenvironment in poly SUS molecular micelles. Comparison of the elution order of benzene derivatives revealed noteworthy selectivity differences between surfactant systems. Log *K* data indicate that the retention in MEKC is primarily due to the hydrophobic interaction between the surfactant system and solutes; however, the effect of the other parameters (e.g., acidity) on retention is very minimal.

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