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Monomeric and polymeric anionic gemini surfactants and mixed surfactant systems in micellar electrokinetic chromatography. Part I: Characterization and application as novel pseudostationary phases

Sodium di(undecenyl) tartarate monomer (SDUT), a vesicle-forming amphiphilic compound possessing two hydrophilic carboxylate head groups and two hydrophobic undecenyl chains gemini surfactant, was prepared and polymerized to form a polymeric gemini surfactant (*i.e.*, poly-SDUT). These anionic surfactant systems with carboxylate (SDUT and poly-SDUT) and sulfate (sodium dodecyl sulfate, SDS) head groups as well as mixed surfactant systems (SDS/SDUT, SDS/poly-SDUT, and SDUT/poly-SDUT) were then applied as novel pseudostationary phases in micellar electrokinetic chromatography (MEKC). The SDUT and poly-SDUT were characterized using various analytical techniques. Retention factors of 36 benzene derivatives were calculated in 20 mM phosphate buffer of each surfactant system. The retention factor values of the test solutes show that there are distinctive selectivity differences between the surfactant systems. Solute-pseudostationary phase interactions in MEKC were also examined by determining the free energy of transfer of the substituted functional groups from the aqueous buffer phase into the pseudostationary phase.

Keywords: Aggregation number / Critical aggregation concentration / Gemini surfactants / Micellar electrokinetic chromatography / Polymeric surfactants DOI 10.1002/elps.200406163

1 Introduction

Capillary electrophoresis (CE) of nonionic solutes cannot be performed in a free solution due to the lack of electric charges of solutes. This problem can be overcome by employing a charged additive that forms micelles that can be used as a pseudostationary phase in fused-silica capillary columns [1, 2]. Sodium dodecyl sulfate (SDS) has been widely used as a micelle-forming additive (*i.e.*, pseudostationary phase) in micellar electrokinetic chromatography (MEKC). In addition, several different types of anionic surfactants, such as bile salts [3, 4], as well as some double chain surfactants, such as disodium 5,12-bis(dodecyloxymethyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulfate [5], sodium dioctyl sulfosuccinate [6–8], and di(2-ethylhexyl) phosphate [9], have been also used in MEKC as pseudostationary phases. As alternatives to

conventional micelles, polymeric surfactants [10–21] offer a simple and convenient way to organize surfactant monomers in MEKC.

Since its first introduction by Terabe [1, 2], MEKC has been extensively used for the separation of both charged and uncharged solutes. A major advantage of MEKC over most separation techniques is the feasibility of changing the chemical composition of the MEKC system by simply rinsing the capillary with a solution of a new pseudostationary phase. Thus, the selectivity of the technique can be easily manipulated and controlled by proper selection of the surfactant type or by addition of modifiers, such as organic solvents or cyclodextrins [22, 23]. In MEKC, uncharged solutes are separated based on their differential partitioning into the pseudostationary phase. The hydrophobic interaction between solutes and the pseudostationary phase is a major driving force behind the solute retention in MEKC. However, some other types of interactions between solutes and the pseudostationary phase also influence solute retention and selectivity. Therefore, understanding the nature of the interactions is critical. Since the retention prediction and selectivity optimization are very critical in rapid method development in MEKC, it is important to achieve a better understanding of the factors that control selectivity.

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Abbreviations: CAC, critical aggregation concentration; poly-SDUT, poly sodium di(10-undecenyl) tartarate; SDUT, sodium di(10-undecenyl) tartarate

Gemini surfactants (also known as dimeric amphiphiles) are made up of two long hydrocarbon chains and two ionic head groups linked by a spacer. These types of surfactants were first introduced by Bunton *et al.* in 1971 [24]. Gemini surfactants have distinct surface and bulk properties that differ from those observed for single-chain conventional surfactants having the same hydrocarbon tail and head group. Conventional surfactants tend to form spherical micelles in dilute solutions, whereas geminis usually form thread-like aggregates. Geminis generally have lower critical aggregation concentration (CAC) values as compared to their counterpart conventional surfactants. In addition, gemini surfactants have excellent foaming and wetting properties, resulting in more efficient lowering of the surface tension of water. They also show unexpected viscosity changes as their concentration is increased [25, 26].

In this first part of our study, a gemini surfactant possessing two hydrophilic carboxylate head groups and two hydrophobic undecenyl chains, sodium di(10-undecenyl) tartarate (SDUT), was prepared and then polymerized using ^{60}Co γ -radiation to form a polymeric gemini surfactant (*i.e.*, poly-SDUT). SDUT and poly-SDUT were characterized using various analytical techniques. These two surfactant systems with carboxylate head groups and a conventional surfactant with sulfate head group (*i.e.*, SDS) as well as mixed surfactant systems of these two types of surfactants (*i.e.*, SDS/SDUT, SDS/poly-SDUT, and SDUT/poly-SDUT) were then applied as novel pseudostationary phase in MEKC. In the second part of this study, a linear solvation energy relationship model was applied for the characterization of retention and selectivity differences between aforementioned pseudostationary phases using MEKC [27].

2 Materials and methods

2.1 Instrumentation

All MEKC experiments were performed on a Beckman (Fullerton, CA, USA) P/ACE model 5510 capillary electrophoresis (CE) instrument equipped with a 0–30 kV power supply, a 21-position inlet and 10-position outlet sample carousel for automatic sample/buffer change, 200, 214, 254, and 280 nm selectable wavelength filters for UV detection, a liquid-thermostated capillary cartridge (capillary, 50 μm ID \times 375 μm OD \times 67 cm total length, 60 cm to the detector), and software System Gold for system control and data handling. The capillary in the Beckman instrument was thermostated by use of a fluoro-organic fluid. The detector time constant was 0.2 s. The detector was operated at 200 nm for benzene derivatives and at

254 for homologues series of alkyl phenyl ketones. All experiments were carried out at 25°C. A voltage of 25 kV was applied throughout the experiments.

2.2 Chemical reagents

All benzene derivatives, SDS, 10-undecenoyl chloride, DL-tartaric acid, and alkyl phenyl ketone homologues, were purchased from Aldrich (Milwaukee, WI, USA). Sodium hydrogenphosphate and sodium dihydrogenphosphate were obtained from EM Science (Gibbstown, NJ, USA). Deionized water was obtained by a water purification system from Millipore (Milford, MA, USA).

2.3 Synthesis of monomeric and polymeric SDUT

The monomer of SDUT was prepared according to a procedure reported by Kunitake and Okahata [28] (Fig. 1). Briefly, 0.04 mol 10-undecenoyl chloride (a) in toluene was added to a pyridine solution of 0.02 mol DL-tartaric acid (b) at 10°C over a period of 30 min. The mixture was

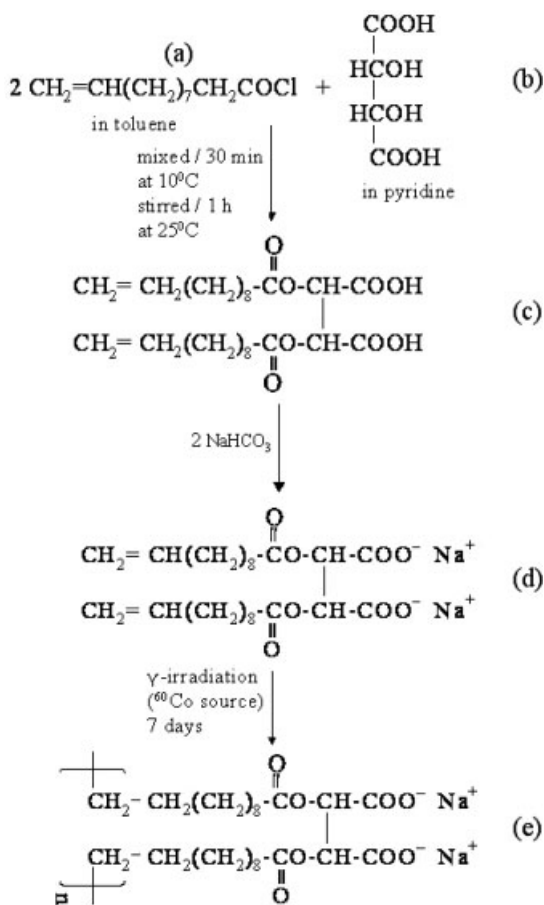


Figure 1. Synthesis scheme for SDUT and poly-SDUT.

then stirred vigorously at room temperature for 1 h. The precipitates formed were filtered and the solution containing the product was concentrated. The resulting solid product, *i.e.*, di(undecylenic) tartaric acid (DUTA) (c), was recrystallized twice from hexane. To prepare the sodium salt of DUTA (*i.e.*, SDUT), a desired amount of DUTA (0.02 mol) was placed in aqueous sodium bicarbonate solution (0.04 mol). The cloudy mixture was stirred until the acidic product (*i.e.*, DUTA) was completely neutralized by alkaline solution. After the mixture turned into a clear solution, which indicated the completion of the titration, the SDUT surfactant solution was freeze-dried to yield a white powder. Recrystallization was performed by dissolving the white powder in an aqueous methanol solution and refrigerated. The crystals were dried in a vacuum desiccator overnight. The final product was SDUT monomers (d). Polymerization of the SDUT monomers (e) was achieved by preparing a 20 mM solution of the surfactant in water and exposing to a ^{60}Co -ray source (~ 680 rad/h) for a week. After radiation, the solution was filtered and lyophilized to yield a white powder. Polymerization was confirmed by the disappearance of the double bond using nuclear magnetic resonance and infrared spectroscopic methods.

2.4 Characterization of the surfactant systems

2.4.1 Determination of SDUT CAC

A surface tension method was used to determine the CAC of the monomeric SDUT surfactant. A 30 mM stock solution of SDUT surfactant was prepared in deionized water (18 M Ω). Twelve different concentrations ranging from 1.0 to 30.0 mM were prepared from this stock solution. A Du Nüoy type tensiometer was used to measure the surface tension. The measured surface tension values were plotted against the surfactant concentration (Fig. 2). The CAC was determined as the crossing point of the two straight lines that fit the experimental values before and after the abrupt change of slope.

2.4.2 Determination of aggregation number

The aggregation number (N) of the surfactants was determined by a fluorescence quenching method proposed by Turro and Yekta [29], using the following expression:

$$\ln\left(\frac{I_0}{I}\right) = \frac{N[Q]}{[S_{\text{tot}}] - \text{CAC}} \quad (1)$$

where I_0 and I are the emission intensities at a certain wavelength in the absence and presence of the quencher, respectively. The concentration of the quencher, *i.e.*,

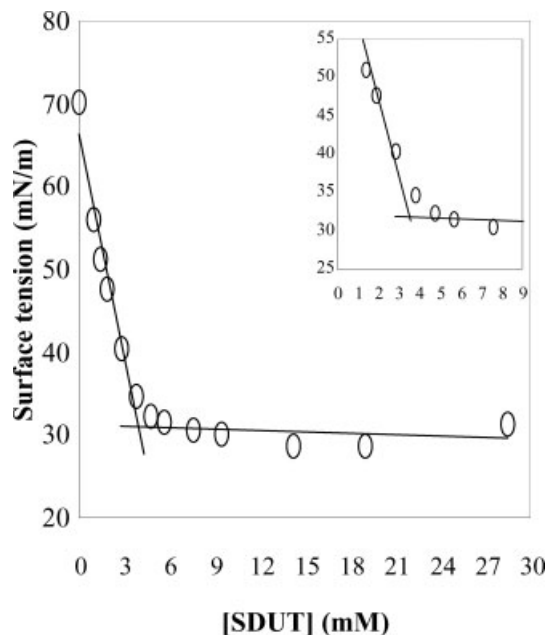


Figure 2. Variation of the surface tension with concentration of SDUT in aqueous solution at room temperature. The inset is an enlargement of the region of interest.

cetylpyridinium chloride (CPyrCl), is represented by [Q]. $[S_{\text{tot}}]$ is the total surfactant concentration and CAC is the critical aggregation concentration of the surfactant. Fluorescence measurements were performed on a SPEX model F2T211 spectrophotometer. Pyrene and CPyrCl were used as fluorescent probe and quencher, respectively. A 1×10^{-3} M stock solution of pyrene was prepared in methanol. A 2.8×10^{-3} M stock solution of CPyrCl and 1.5% w/v of each of SDS, SDUT, and poly-SDUT stock solutions were prepared separately in deionized water. A known volume of pyrene stock solution was pipetted into a clean volumetric flask; methanol was evaporated by nitrogen gas and aqueous surfactant solution was added. At this step, the concentrations of pyrene and each surfactant were 2.0×10^{-5} M and 1.5% w/v, respectively (solution 1). After sonicating for 90 min, solution 1 was stored in a dark area overnight to equilibrate. Solution 1 was then divided in half. One half was diluted with deionized water to give a 1.0×10^{-5} M pyrene and 0.75% w/v surfactant (solution 2), while the other half was mixed with quencher stock solution to make 1.4×10^{-3} M CPyrCl, 1.0×10^{-5} M pyrene, and 0.75% w/v surfactant (solution 3). Solution 3 was added to solution 2 in increasing increments of 25 μL and allowed 20 min for equilibration after addition of each quencher solution before fluorescence measurement. The decrease in emission spectra of pyrene was recorded at 393.0 nm after each aliquot of the quencher solution (solution 3) was added and the logarithm of the intensity ratio I_0/I was

plotted against the quencher concentration. N is obtained from the slope of the plot of $\ln(I_0/I)$ vs. $[Q]$ (i.e., $N = \text{slope} \times ([S_{\text{tot}}] - \text{CAC})$). A representative N measurement plot for poly-SDUT is shown in Fig. 3.

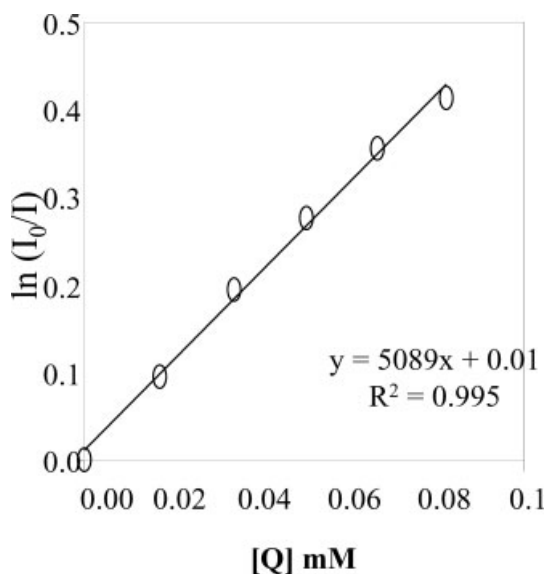


Figure 3. Degree of aggregation measurement for (A) SDS, (B) SDUT, and (C) poly-SDUT.

2.4.3 Determination of the polarity of the surfactant systems

The polarity of the aggregated surfactant can be measured using a fluorescence molecule that stays in the surfactant aggregate and is sensitive to the polarity of the microenvironment. Pyrene is a fluorescent molecule that has been extensively used for this purpose. The emission spectrum of the pyrene molecule is sensitive to the environment in which it is dissolved. Pyrene has characteristic fluorescence emission spectra that consist of five vibronic bands. The intensities of these vibronic bands depend on the polarity of the environment in which pyrene is dissolved. An increase in the intensity of the band I at 372 nm is accompanied by a decrease in the intensity of the band III at 383 nm with increasing polarity of the environment. The ratio of the intensity of band I to band III is often used to determine the polarity of the aggregated surfactant core.

2.5 Capillary electrophoresis procedure

All new capillaries were activated by the following washing sequence: 1 h 1 M NaOH and 20 min of triply deionized water. Prior to each separation with the same surfactant system, the capillary was rinsed with triply deionized water (5 min), 0.1 M NaOH (3 min), and separation buffer

(3 min). When the surfactant was changed, the capillary was reconditioned for 15 min with deionized water, 10 min with 0.1 M NaOH, and 5 min with the separation buffer. Unless otherwise noted, the time for pressure injection was 3 s for most separations by applying 0.5 psi pressure.

2.6 Preparation of separation buffers and standard solutions

A 100 mM stock solution of phosphate buffer (pH 7.0) was prepared by dissolving appropriate amounts of sodium hydrogenphosphate and sodium dihydrogenphosphate and refrigerated after each use. The solutions of SDS, SDUT, and poly-SDUT were prepared by first dissolving 0.1 g surfactant in 5 mL deionized water. Two mL of a 100 mM phosphate stock buffer was then added to this solution. Lastly, the final volume was adjusted to 10 mL with deionized water. The same sequence was followed for the preparation of mixed surfactant solutions except 0.05 g of each surfactant (e.g., 0.05 g SDS and 0.05 g SDUT for SDS/SDUT mixed surfactant system) was dissolved in 5 mL of water to keep final surfactant concentration at 1.0% w/v. The corresponding molar concentrations of the pseudostationary phases were 34.7 mM SDS, 19 mM SDUT, and 19 mM equivalent monomer concentration of poly-SDUT. The mixed surfactants contained 17.35 mM SDS and 9.5 mM SDUT or poly-SDUT for SDS/SDUT or SDS/poly-SDUT, and 9.5 mM each of SDUT and poly-SDUT for SDUT/poly-SDUT mixture. After a thorough mixing in a sonicator for 10 min, the final running buffers were filtered through a 0.45 μm syringe filter (Nalgene, Rochester, NY, USA), then degassed for 3 min before capillary electrophoretic experiments. All stock solute solutions were prepared in methanol at concentrations of ca. 4–7 mM each. Molar concentrations of the injected standard solute mixture were about 0.5 mM.

2.7 Calculations

The retention factor, k' , of a neutral solute was measured according to the following equation [2]:

$$k' = \frac{t_R - t_{\text{eo}}}{t_{\text{eo}} \left(1 - \frac{t_R}{t_{\text{psp}}} \right)} \quad (2)$$

where t_R , t_{eo} , and t_{psp} are the migration times of a neutral retained solute, the electroosmotic flow (EOF), and the pseudostationary phase, respectively. Methanol was used as the t_{eo} marker and was measured from the time of injection to the first deviation from the baseline. Decanophenone was used as tracer for t_{psp} . The elution range is defined as $t_{\text{psp}}/t_{\text{eo}}$.

The apparent electrophoretic mobility of the pseudostationary phase (μ_{app} , $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$) was calculated by Eq. (3):

$$\mu_{\text{app}} = \frac{I_t I_d}{V t_{\text{psp}}} \quad (3)$$

where I_t is the total length of the capillary (cm), I_d is the length of the capillary from injector to detector (cm), V is the applied voltage (V), and t_{psp} is measured in seconds (s). To calculate the electroosmotic mobility of the buffer solution (μ_{eo} , $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$), the t_{psp} term in Eq. (3) is replaced with t_{eo} . The effective electrophoretic mobility (μ_{ep}) of each surfactant system was calculated from their net electrophoretic velocity (v_{net} , $\text{cm} \cdot \text{s}^{-1}$) values [30]:

$$v_{\text{net}} = v_{\text{ep}} - v_{\text{eo}} = \frac{I_d}{t_{\text{psp}}} - \frac{I_d}{t_{\text{eo}}} \quad (4)$$

$$\mu_{\text{ep}} = \frac{v_{\text{net}} I_t}{V} \quad (5)$$

where v_{ep} and v_{eo} are the electrophoretic velocity and electroosmotic velocity of the pseudostationary phase, respectively. The methylene (or hydrophobic) selectivity, α_{CH_2} , was calculated from the antilogarithm of the slope of the regression line of $\log k'$ vs. carbon number of alkyl phenyl ketone homologous series.

3 Results and discussion

3.1 Physicochemical properties of pseudostationary phases

The primary structural difference between pseudostationary phases used in this study is their head groups. Surfactant systems with carboxylate (SDUT, poly-SDUT, SDUT/poly-SDUT), sulfate (SDS), and the mixture of these two (SDS/SDUT, SDS/poly-SDUT) head groups were examined. Sodium is a counterion and the alkyl chain (C11 for SDUT and poly-SDUT, C12 for SDS) is their hydrophobic moiety for all surfactants. The physicochemical properties of the pseudostationary phases are compared in Table 1. The N value of SDUT (275 monomers per aggregate) is higher than that of SDS (71 monomers per micelle) and poly-SDUT (71 monomer per aggregate). Possessing double hydrophobic chains and two head groups, SDUT and poly-SDUT tend to form vesicles [28] or thread-like aggregates [26]. After polymerization of SDUT, the number of aggregates is decreased resulting in a smaller N value for poly-SDUT. This may be due to the slower and continuous rearrangement of the monomers in the aggregates during the long polymerization process (7 days). The flux of the γ -rays from ^{60}Co (0.7 krad/h) was probably not strong enough to provide polymer aggregates with N values similar to that of the monomer aggregates. A shorter polymerization time with

a stronger radiation source may result in higher N values of poly-SDUT. Previous studies have shown that the intensity of the radiation source used for polymerization has a significant effect on the number of the repeat units of polymers [31]. Using a relatively stronger γ -radiation source (143 krad/h), Paleos *et al.* [32] have obtained polymers with the same size as the monomeric micelles by polymerization of sodium 10-undecenoate.

As shown in Table 1, the SDS system has the highest μ_{eo} value ($5.60 \times 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$). The μ_{eo} value is smallest for SDS/SDUT and SDUT/poly-SDUT systems, which have the same value of $4.77 \times 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$. The difference in μ_{eo} values for each pseudostationary phase is probably due to the difference in zeta potentials of the capillary wall, of the pseudostationary phase, and the difference in viscosity of the separation buffer. The μ_{app} value is the highest for SDS micelles while it is the lowest for SDS/SDUT aggregates among all surfactant systems. Since all surfactant systems are negatively charged, v_{eo} is higher than v_{ep} ; hence, v_{net} and μ_{ep} will take negative values (Table 1). The signs indicate the relative direction of μ_{ep} . In the present case, all pseudostationary phases move toward anode (opposite direction of EOF). In practice, the negative sign is often neglected. As seen from Table 1, SDS has the highest μ_{ep} , whereas SDUT/poly-SDUT has the lowest μ_{ep} value.

The methylene selectivity (α_{CH_2}) measurements indicate that gemini surfactants have a more hydrophobic character than the SDS micellar phase under the experimental conditions studied. SDUT is the most ($\alpha_{\text{CH}_2} = 3.31$) and SDS is the least ($\alpha_{\text{CH}_2} = 2.57$) hydrophobic phase. In contrast, fluorescence polarity measurement indicated that SDUT was the most polar surfactant probably due to the fact that pyrene, the fluorescent probe used in polarity measurement, is dissolved in the relatively polar region of the SDUT aggregates. It should be mentioned that vesicles are spherically closed bilayers, which, in analogy to the cell membranes, enclose an aqueous compartment [33, 34]. When pyrene is dissolved in or near this polar aqueous region, the ratio of band I (at 372 nm) to band III (at 383 nm) intensities of pyrene's spectra increases, which indicates a more polar environment.

3.2 Application of pseudostationary phases in MEKC

The retention behavior of 36 test solutes (Table 2) in each pseudostationary phase was examined and the retention factors of the test solutes were calculated by Eq. (2). Based on their hydrogen bond-donating or -accepting abilities, the solutes in Table 2 can be characterized as nonhydrogen bond donors (NHBs) (solutes 1–12), hydro-

Table 1. Comparison of the physicochemical properties of six pseudostationary phases

Physicochemical property	Pseudostationary phase					
	SDS	SDUT	Poly-SDUT	SDS/SDUT	SDS/poly-SDUT	SDUT/poly-SDUT
Degree of aggregation ^{a)} , N	71	275	71	na ^{b)}	na	na
Critical aggregation concentration, CAC (mM)	8.00 ^{c)}	3.94 ^{d)}	0.00 ^{e)}	na	na	na
Electroosmotic mobility ^{f), g)} , μ_{eo} ($10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$)	5.60	4.79	4.97	4.77	5.09	4.77
Apparent electrophoretic mobility ^{f), h)} , μ_{app} ($10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$)	1.55	1.18	1.32	1.13	1.23	1.26
Effective electrophoretic mobility ^{f), i)} , μ_{ep}	4.05	-3.61	-3.65	-3.64	-3.86	-3.51
($10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$)						
Methylene-group selectivity ^{f), j)} , α_{CH_2}	2.57	3.31	2.72	2.97	2.78	2.71
Migration-time window ^{f)} , t_{psp}/t_{eo}	3.61	4.06	3.77	4.22	4.14	3.80
Polarity (pyrene I/III) ^{k)}	1.09	1.31	0.98	1.05	1.04	0.97

a) Determined in water by fluorescence quenching method

b) Data not available

c) From [32]

d) Determined in water by surface tension measurement

e) CAC of the polymerized surfactant is assumed to be zero.

f) Data were collected with a 67 cm (60 cm effective length) \times 50 μm ID capillary with an applied voltage of +25 kV using a 20 mM phosphate buffer at pH of 7.0; final surfactant concentration, 1.0% w/v.g) Calculated using Eq. (3), t_{psp} was replaced with t_{eo} .

h) Calculated using Eq. (3)

i) Calculated using Eq. (5)

j) Calculated from the antilogarithm of the slope of the regression line of $\log k'$ vs. carbon number of alkyl phenyl ketones (C10-C14)

k) Determined from the ratio of the intensity of band I and band III of pyrene in the presence of 0.75% w/v surfactant using fluorescence spectroscopy

Table 2. Test benzene derivatives used in this study

NHBS		HBAs		HBDs	
1	Benzene	13	Acetophenone	25	Benzyl alcohol
2	Toluene	14	Benzonitrile	26	Phenol
3	Ethylbenzene	15	Nitrobenzene	27	4-Methylphenol
4	Propylbenzene	16	Methyl benzoate	28	4-Ethylphenol
5	<i>p</i> -Xylene	17	Ethyl benzoate	29	4-Fluorophenol
6	Chlorobenzene	18	4-Chloroanisole	30	4-Chlorophenol
7	Bromobenzene	19	4-Nitrotoluene	31	4-Bromophenol
8	Iodobenzene	20	4-Chloroacetophenone	32	4-Chloroaniline
9	4-Chlorotoluene	21	Methyl 2-methylbenzoate	33	3-Chlorophenol
10	Biphenyl	22	Phenyl acetate	34	3-Methylphenol
11	Naphthalene	23	3-Methylbenzyl alcohol	35	3-Bromophenol
12	1-Methylnaphthalene	24	Phenethyl alcohol	36	3,5-Dimethylphenol

gen bond acceptors (HBAs) (solutes 13–24), and hydrogen bond donors (HBDs) (solutes 25–36). The NHB solutes that include alkyl- and halo-substituted benzenes and polyaromatic hydrocarbons do not have any hydrogen-bonding functional groups; however, due to the aromatic ring(s), they are weak HBAs. The HBA solutes possess hydrogen bond-accepting functional groups on the aromatic ring, whereas HBD solutes have hydrogen bond-donating functional groups. Solute interactions with the vesicular and micellar systems occur through a variety of mechanisms, such as surface adsorption, coaggregation, or partitioning into the hydrophobic core of micelles or vesicles. Due to these different mechanisms, the retention factors of the test solutes in each pseudostationary phase system are expected to be different if pseudostationary phases have different selectivity.

3.3 Comparison of retention factors for different pseudostationary phases

The different retention factors of the test solutes in each pseudostationary phase indicate that the overall migration patterns in all six surfactant systems are different. This dissimilarity in migration pattern can be confirmed by plotting the logarithm of retention factors of the test solutes in each surfactant system against each other. In Fig. 4, similarities and differences in retention behaviors of pseudostationary phases are compared with that of SDS. Relatively higher correlations between the migration patterns of the surfactant systems are obvious due to the fact that hydrophobic interaction are the major factors governing solute-pseudostationary phase interaction.

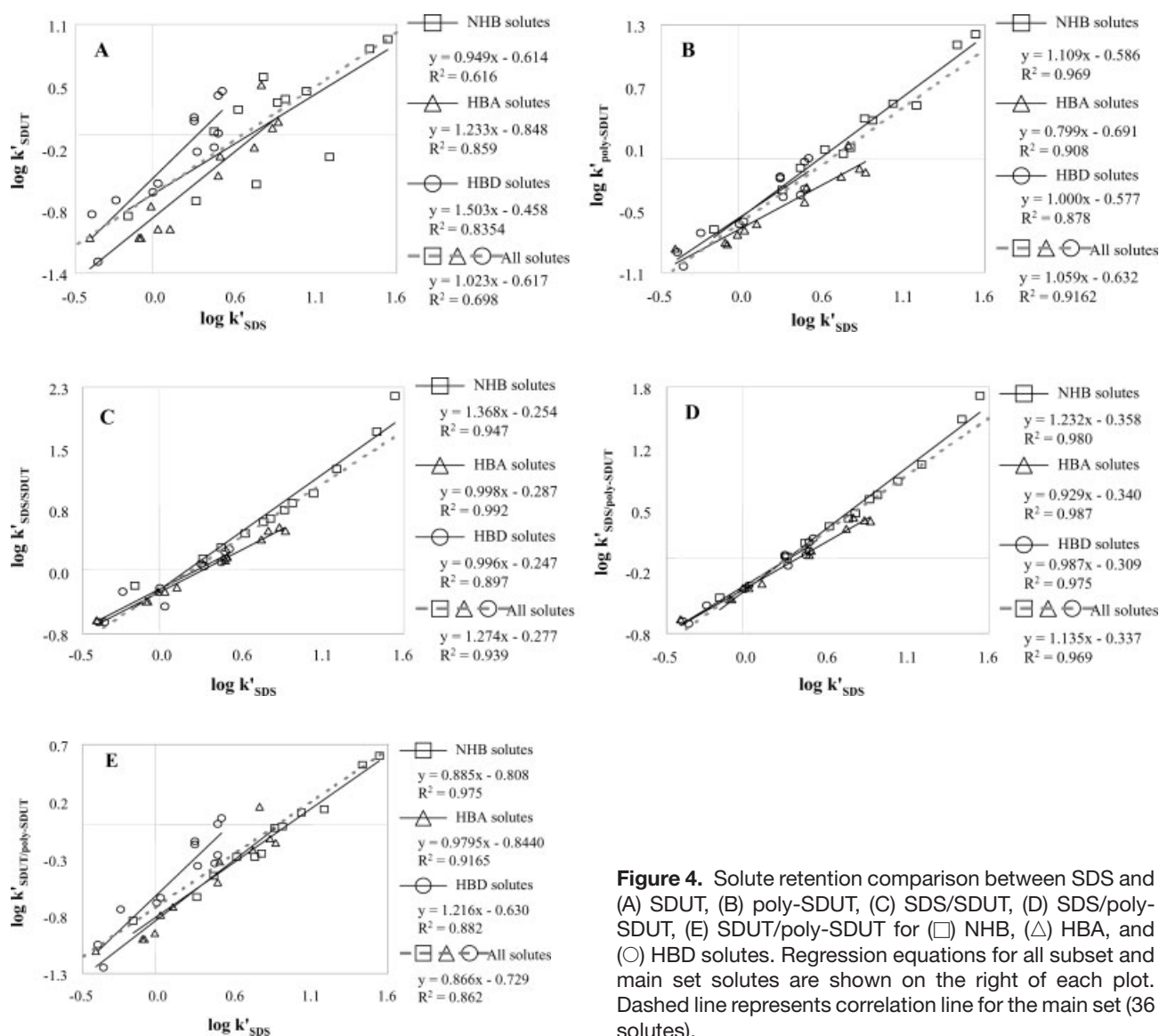


Figure 4. Solute retention comparison between SDS and (A) SDUT, (B) poly-SDUT, (C) SDS/SDUT, (D) SDS/poly-SDUT, (E) SDUT/poly-SDUT for (□) NHB, (△) HBA, and (○) HBD solutes. Regression equations for all subset and main set solutes are shown on the right of each plot. Dashed line represents correlation line for the main set (36 solutes).

The carboxylate head group of SDUT and poly-SDUT are weaker Brønsted-Lowry acids (or relatively stronger bases) as compared to the sulfate headgroup of SDS. Figure 4A confirms that the HBD solutes show a clear deviation above the dashed line, which represents the linear regression data for the main solute set, indicating stronger interaction between the HBD solutes and SDUT system. The HBA bases, however, show deviation below the dashed line, indicating stronger interactions with the SDS micelles. This shows that HBA bases find the SDS micellar environment more acidic than the SDUT system or bulk aqueous solution. The majority of the NHB solutes show similar affinity (*i.e.*, fall on or near the dashed line) for both SDUT and SDS. Three solutes, *i.e.*, *p*-xylene, chlorobenzene, and bromobenzene, interact strongly with SDUT while toluene, ethylbenzene, and propylbenzene show stronger affinity for the SDS system.

Poly-SDUT shows weaker basic characteristic than SDUT, probably due to a smaller aggregation number, resulting in a relatively lower number of carboxylate head groups on the surface of its vesicles. In addition, possible partial hydrolysis of carboxylate head groups during the polymerization process and/or the change in the structural configuration of poly-SDUT may also effect the decrease of basicity. When compared to SDS, poly-SDUT interacts relatively more strongly with HBD acidic solutes (Fig. 4B). However, a few HBD solutes fall below the dashed line (*i.e.*, favor SDS). As will be discussed in the second part of this study [27], SDS provides a marginally more dipolar microenvironment than poly-SDUT, thus polar HBD solutes prefer SDS than relatively less polar poly-SDUT phase.

As seen in Figs. 4C and D, NHB and HBD solutes have similar affinity for SDS/SDUT and SDS/poly-SDUT mixed surfactant systems. The HBD solutes show minor deviations below the dashed line (Fig. 4C), indicating a slightly stronger interaction with SDS than SDS/SDUT. The correlation between SDS and SDS/poly-SDUT in Fig. 4D shows that the majority of the HBD solutes fall on the line, indicating similar affinity for both surfactant systems. The HBA bases find SDS micelles more acidic than SDS/SDUT and SDS/poly-SDUT mixed systems (Figs. 4C and D). Therefore, HBA bases show deviations below the dashed line. In contrast, the NHB solutes fall on the line with only a few exceptions indicating similar selectivity of these three surfactant systems toward hydrophobic solutes.

Similar to SDUT, the SDUT/poly-SDUT mixed surfactant system is a strong HBA [27], hence, interacts more strongly with HBD acids (Fig. 4E). The HBA bases, however, have stronger affinity for SDS than SDUT/poly-SDUT due to the relatively stronger acidic character of the for-

mer. This is shown in Fig. 4E where HBA solutes show noticeable deviations below the dashed line. Due to their nonpolar character, SDS micelles interact strongly with NHB solutes. Correlation between SDUT and poly-SDUT reveals that NHB solutes interact with poly-SDUT stronger, while HBA and HBD solutes prefer SDUT (plots not shown). When SDUT and SDS/SDUT surfactants are compared, NHB solutes tend to retain in the latter and HBD in the former; however, these two surfactant systems have similar selectivity toward HBA solutes. Similarly, HBD solutes have high affinity for SDUT, whereas HBA solutes prefer SDUT/poly-SDUT. The NHB solutes show no differences in selectivity in both surfactant systems. Poly-SDUT and SDS/SDUT surfactant systems show almost the same selectivity towards all solute sets.

The log k' values of the main set, NHB, HBA, and HBD solutes for each surfactant system are plotted against each other, and R^2 , slope, and the y -intercept values for each correlation line are listed in Tables 3 and 4. As seen in Table 3 (white background), the selectivity of SDS/poly-SDUT mixed surfactant is very similar to that of SDS, poly-SDUT, and SDS/SDUT surfactant systems (higher R^2 values) toward the complete set of solutes. Poly-SDUT, SDS/poly-SDUT, and SDUT/poly-SDUT systems have similar selectivity toward NHB solutes (Table 3, dark shaded background). Likewise, SDS/SDUT and SDS/poly-SDUT surfactant systems have also similar selectivity toward NHB solutes. Major selectivity differences (*i.e.*, lower R^2 value) are observed when log k' values of the complete solute set (white background) or NHB solutes (dark background) are compared using SDUT and poly-SDUT, SDS/SDUT, or SDS/poly-SDUT (Table 3). Highest similarity in selectivity toward HBA basic solutes are seen between SDS/SDUT vs. SDS/poly-SDUT mixed surfactant systems. Similar resemblance is observed between SDS vs. SDS/SDUT, SDS vs. SDS/poly-SDUT, and poly-SDUT vs. SDUT/poly-SDUT surfactant systems. On the contrary, different selectivity is observed for the same solute set when SDS and SDUT or poly-SDUT is compared (Table 4, white background). SDUT/poly-SDUT and poly-SDUT surfactants are found to have quite similar selectivity, whereas SDS and SDUT have different selectivity toward the HBD acidic solutes (Table 4, dark background).

3.4 Energy of transfer determination for functional group selectivity

Solute-pseudostationary phase interactions in MEKC can also be examined by determining the free energy of transfer ($\Delta\Delta G$) of the substituted functional groups from the aqueous buffer phase into the pseudostationary phase. The functional group selectivity, τ , can be defined

Table 3. R^2 , slope, and y-intercept values of log k' comparison plots of complete solute set (white background) and of 12 NHB solutes (shaded background)

Surfactant systems		SDS	SDUT	Poly-SDUT	SDS/SDUT	SDS/ poly-SDUT	SDUT/ poly-SDUT
SDS	R^2	1.00	0.62	0.97	0.95	0.98	0.98
	Slope	1.00	0.95	1.11	1.37	1.23	0.89
	Intercept	0.00	-0.61	-0.59	-0.25	-0.36	-0.81
SDUT	R^2	0.70	1.00	0.70	0.58	0.63	0.67
	Slope	1.02	1.00	0.78	0.89	0.82	0.61
	Intercept	-0.62	0.00	0.18	0.70	0.50	-0.20
Poly-SDUT	R^2	0.92	0.77	1.00	0.96	0.99	0.99
	Slope	1.06	0.79	1.00	1.22	1.10	0.79
	Intercept	-0.63	-0.04	0.00	0.47	0.30	-0.34
SDS/SDUT	R^2	0.94	0.66	0.96	1.00	0.99	0.96
	Slope	1.27	0.87	1.16	1.00	0.88	0.62
	Intercept	-0.28	0.41	0.48	0.00	-0.11	-0.62
SDS/poly-SDUT	R^2	0.97	0.71	0.97	0.99	1.00	0.98
	Slope	1.14	0.79	1.03	0.87	1.00	0.71
	Intercept	-0.34	0.28	0.33	-0.09	0.00	-0.55
SDUT/poly-SDUT	R^2	0.86	0.87	0.89	0.82	0.86	1.00
	Slope	0.87	0.71	0.80	0.64	0.75	1.00
	Intercept	-0.73	-0.24	-0.22	-0.53	-0.47	0.00

The R^2 , slope, and y-intercept values listed in lightly shaded area represent the ideal values when no selectivity difference is observed between compared two surfactant systems.

Table 4. The R^2 , slope, and y-intercept values of log k' comparison plots of 12 HBA solutes (white background) and of 12 HBD solutes (shaded background)

Surfactant systems		SDS	SDUT	Poly-SDUT	SDS/SDUT	SDS/ poly-SDUT	SDUT/ poly-SDUT
SDS	R^2	1.00	0.84	0.88	0.90	0.98	0.88
	Slope	1.00	1.50	1.00	1.00	0.99	1.22
	Intercept	0.00	-0.46	-0.58	-0.25	-0.31	-0.63
SDUT	R^2	0.86	1.00	0.99	0.87	0.93	0.98
	Slope	1.23	1.00	0.65	0.60	0.59	0.78
	Intercept	-0.85	0.00	-0.28	0.04	-0.03	-0.27
Poly-SDUT	R^2	0.91	0.97	1.00	0.91	0.96	0.99
	Slope	0.80	0.62	1.00	0.94	0.92	1.21
	Intercept	-0.69	-0.16	0.00	0.30	0.23	0.07
SDS/SDUT	R^2	0.99	0.91	0.94	1.00	0.93	0.92
	Slope	1.00	0.72	1.16	1.00	0.92	1.18
	Intercept	-0.29	0.35	0.54	0.00	-0.07	-0.03
SDS/poly-SDUT	R^2	0.99	0.92	0.96	1.00	1.00	0.96
	Slope	0.93	0.68	1.09	0.93	1.00	1.27
	Intercept	-0.34	0.26	0.43	-0.07	0.00	-0.24
SDUT/poly-SDUT	R^2	0.92	0.94	0.99	0.94	0.96	1.00
	Slope	0.98	0.74	1.22	0.99	1.07	1.00
	Intercept	-0.84	-0.20	0.00	-0.56	-0.49	0.00

The R^2 , slope, and y-intercept values listed in lightly shaded area represent the ideal values when no selectivity difference is observed between compared two surfactant systems.

as the ratio of the k' of a substituted benzene (Ph-R) over the k' of benzene (Ph-H):

$$\tau = \frac{k'_{\text{Ph-R}}}{k'_{\text{Ph-H}}} \quad (6)$$

Then $\Delta\Delta G$ can be calculated by Eq. (7):

$$\Delta\Delta G = -RT \ln \tau \quad (7)$$

where R is the universal gas constant (8.31451 J/mol K) and T is the absolute temperature ($0^\circ\text{C} = 273.15 \text{ K}$).

The $\Delta\Delta G$ values for various functional groups are listed in Table 5. A negative $\Delta\Delta G$ value indicates that the addition of a functional group to benzene ring leads to an increase in interaction with the pseudostationary phase. The larger negative $\Delta\Delta G$ indicates more favorable interactions between pseudostationary phase and the substituted solute as compared to that between pseudostationary phases and the unsubstituted benzene molecule. As seen in Table 5, the $\Delta\Delta G$ values of the NHB functional groups (solutes 1–7) are all negative in all surfactant systems, as the hydrophobicity and the size of the functional group increases the interaction with the pseudostationary phase increases accordingly. Particularly, the negative value of $\Delta\Delta G$ increases as the carbon number of the functional group (solutes 1–4) increases. A similar trend is observed for halogen functional groups (solutes 5–7). It is interesting to note that SDUT provides less negative $\Delta\Delta G$ values for solutes 1–3 in Table 5, while more negative $\Delta\Delta G$ values for solutes 5–7 as compared to the remaining surfactant systems.

The HBA functional groups (solutes 8–13) show relatively stronger interactions (more negative $\Delta\Delta G$ values) with the SDS system. Larger and more hydrophobic functional groups (solutes 10–12) have favorable interactions with all surfactant systems. It should be noted that alcohol functional groups have positive $\Delta\Delta G$ values in all pseudostationary phases except solute 13, which has a slightly negative $\Delta\Delta G$ value in SDS. The strongest interaction between phenol (solute 14) and a pseudostationary phase is observed for the SDUT surfactant system (negative $\Delta\Delta G$ value) as compared to the rest of the surfactant systems (positive $\Delta\Delta G$). Since phenol is a HBD acid, SDUT acts like a strong basic surfactant. The weakest interaction occurs between phenol and the SDS/SDUT system (larger positive $\Delta\Delta G$ value).

4 Concluding remarks

Anionic surfactant systems with carboxylate (SDUT and poly-SDUT) and sulfate (SDS) head groups as well as mixed surfactant systems (SDS/SDUT, SDS/poly-SDUT, and SDUT/poly-SDUT) were applied as pseudostationary phases in MEKC. The SDUT and poly-SDUT were synthesized and characterized using various analytical techniques. The aggregation number of SDUT was found to be higher than that of SDS and poly-SDUT. The SDS system has the highest while the SDS/SDUT and SDUT/poly-SDUT systems have the lowest electroosmotic mobilities. The methylene selectivity measurements show that gemini pseudostationary phases have a more hydrophobic character than SDS micellar phase under the

Table 5. Effect of pseudostationary phases on functional group selectivity

		$\Delta\Delta G = -RT \ln \tau$ (kJ/mol)					
Functional group		SDS	SDUT	Poly-SDUT	SDS/SDUT	SDS/poly-SDUT	SDUT/poly-SDUT
1	-CH ₃	-2.52	-0.83	-2.17	-1.96	-2.48	-1.19
2	-CH ₂ CH ₃	-4.80	-1.77	-4.18	-4.54	-4.79	-3.20
3	-CH ₂ CH ₂ CH ₃	-7.51	-3.37	-6.82	-8.41	-8.03	-5.56
4	-C ₆ H ₅	-9.67	-10.09	-10.78	-13.66	-12.20	-8.22
5	-Cl	-3.20	-4.80	-3.37	-2.74	-3.34	-2.24
6	-Br	-4.09	-6.09	-4.40	-3.75	-4.29	-3.20
7	-I	-5.57	-6.47	-6.12	-5.47	-5.94	-4.63
8	-CN	-0.47	1.31	0.86	1.11	0.10	0.90
9	-NO ₂	-0.85	-0.52	0.30	0.41	-0.56	0.60
10	-C(O)CH ₃	-1.56	0.79	-0.29	0.10	-0.87	-0.70
11	-C(O)OCH ₃	-3.34	-2.30	-1.49	-1.79	-2.58	-1.94
12	-C(O)OCH ₂ CH ₃	-5.61	-5.35	-3.12	-3.97	-4.61	-3.88
13	-CH ₂ CH ₂ OH	-0.39	1.31	0.73	1.11	0.10	0.90
14	-CH ₂ OH	1.15	2.64	2.03	2.62	1.59	2.34
15	-OH	1.35	-0.07	1.27	2.62	1.42	1.17

experimental conditions studied. SDUT and poly-SDUT were found to act as weaker Brønsted-Lowry acids as compared to SDS. Therefore, HBD acidic solutes interact relatively stronger with SDUT and poly-SDUT than SDS. The HBA basic solutes, however, interact stronger with SDS micelles. When SDS and SDS/SDUT or SDS/poly-SDUT systems are compared, SDS shows slightly more basic character than the latter two mixed surfactant systems. The $\Delta\Delta G$ values show that interaction between pseudostationary phase and the solutes increases with an increase in hydrophobicity and the size of the functional group. Halogen substituted solutes interact strongly with SDUT system (*i.e.*, $\Delta\Delta G$ values are relatively more negative in SDUT system). The $\Delta\Delta G$ values also indicate that HBA functional groups show relatively stronger interactions with SDS.

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5 References

- [1] Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., Ando, T., *Anal. Chem.* 1984, 56, 111–113.
- [2] Terabe, S., Otsuka, K., Ando, T., *Anal. Chem.* 1985, 57, 834–841.
- [3] Terabe, S., Shibata, M., Miyashita, Y., *J. Chromatogr.* 1989, 480, 403–411.
- [4] Otsuka, K., Terabe, S., Ando, T., *J. Chromatogr.* 1985, 332, 211–217.
- [5] Tanaka, N., Ishida, T., Araki, T., Masuyama, A., Nakatsuji, Y., Okahara, M., Terabe, S., *J. Chromatogr.* 1993, 648, 469–473.
- [6] Shi, Y., Fritz, J. S., *Anal. Chem.* 1995, 67, 3023–3027.
- [7] Luong, J. H. T., *Electrophoresis* 1998, 19, 723–730.
- [8] Wall, W., Chan, K., El Rassi, Z., *Electrophoresis* 2001, 22, 2320–2326.
- [9] Akbay, C., Shamsi, S. A., Warner, I. M., *Electrophoresis* 1997, 18, 253–259.
- [10] Terabe, S., Ozaki, H., Tanaka, Y., *J. Chin. Chem. Soc.* 1994, 41, 251–257.
- [11] Yang, S. Y., Bumgarner, J. G., Khaledi, M. G., *J. High Resolut. Chromatogr.* 1995, 18, 443–445.
- [12] Tanaka, N., Nakagawa, K., Hosoya, K., Palmer, C. P., Kunugi, S., *J. Chromatogr. A* 1998, 802, 23–33.
- [13] Tanaka, N., Fukutoma, T., Hosoya, K., Kimita, K., Araki, T., *J. Chromatogr. A* 1995, 716, 57–67.
- [14] Shamsi, S. A., Akbay, C., Warner, I. M., *Anal. Chem.* 1998, 70, 3078–3083.
- [15] Akbay, C., Shamsi, S. A., Warner, I. M., *J. Chromatogr. A* 2001, 910, 147–155.
- [16] Akbay, C., Shamsi, S. A., Warner, I. M., *Electrophoresis* 1999, 20, 145–151.
- [17] Palmer, C. P., Terabe, S., *Anal. Chem.* 1997, 69, 1852–1860.
- [18] Palmer, C. P., Khaled, M. Y., McNair, H. M., *J. High Resolut. Chromatogr.* 1992, 15, 756–762.
- [19] Akbay, C., Gill, N. L., Agbaria, R. A., Warner, I. M., *Electrophoresis* 2003, 24, 4209–4220.
- [20] Palmer, C. P., *J. Chromatogr. A* 1997, 780, 75–92.
- [21] Akbay, C., Shamsi, S. A., *Electrophoresis* 2004, 25, 622–634.
- [22] Poole, C. F., Poole, S. K., *J. Chromatogr. A* 1997, 792, 89–104.
- [23] Poole, C. F., Poole, S. K., Abraham, M. H., *J. Chromatogr. A* 1998, 798, 207.
- [24] Bunton, C. A., Robinson, L., Schaak, J., Stam, M. F., *J. Org. Chem.* 1971, 36, 2346–2350.
- [25] Menger, F. M., Littau, C. A., *J. Am. Chem. Soc.* 1993, 115, 10083–10090.
- [26] Jennings, K., Marshal, I., Birrell, H., Edwards, A., Haskins, N., Sodermann, O., Kirby, A. J., Camilleri, P., *Chem. Commun.* 1998, 1951–1952.
- [27] Akbay, C., Agbaria, R. A., Warner, I. M., *Electrophoresis* 2004, 25, 426–445.
- [28] Kunitake, T., Okahata, Y., *Bull. Chem. Soc. Jpn.* 1978, 51, 1877–1879.
- [29] Turro, N. J., Yekta, A., *J. Am. Chem. Soc.* 1978, 100, 5951–5952.
- [30] Kuhn, R., Hoffstetter-Kuhn, S., *Capillary Electrophoresis: Principles and Practice*, Springer-Verlag, Berlin 1993, p. 20.
- [31] Davis, J. E., Senogles, E., *Aust. J. Chem.* 1981, 34, 1413–1417.
- [32] Paleos, C. M., Stassinopoulou, C. I., Malloaros, A., *J. Phys. Chem.* 1983, 87, 251–254.
- [33] Hinze, W. L., Armstrong, D. W. (Eds.), *Ordered Media in Chemical Separation* (ACS Symposium Series, No. 342), American Chemical Society, Washington, DC 1987.
- [34] Fuhrhop, J. H., Mathieu, J., *Angew. Chem. Int. Ed. Engl.* 1984, 23, 100–109.