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Characterization and application of sodium di(2-ethylhexyl) sulfosuccinate and sodium di(2-ethylhexyl) phosphate surfactants as pseudostationary phases in micellar electrokinetic chromatography

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Abstract

Sodium di(2-ethylhexyl) sulfosuccinate (DOSS) and sodium di(2-ethylhexyl) phosphate (NaDEHP) surfactants, with double alkyl chains and negatively charged headgroups, were characterized using fluorescence quenching, densitometry, and tensiometry techniques to determine their aggregation number, partial specific volume, and critical aggregation concentration. These two surfactants were then applied as pseudostationary phases in micellar electrokinetic chromatography (MEKC) for separations of alkyl phenyl ketones. The aggregation number of NaDEHP was found to be more than two-fold higher than that of DOSS. The partial specific volumes of NaDEHP and DOSS were found to be 0.9003 and 0.8371 mL/g, respectively. The critical aggregation concentrations are 5.12 and 1.80 mM for NaDEHP and DOSS, respectively. The DOSS surfactant provided a wider separation window and had a greater hydrophobic environment than the NaDEHP surfactant under the MEKC experimental conditions studied.

Keywords: Micellar electrokinetic chromatography; Sodium di(2-ethylhexyl) sulfosuccinate; Sodium di(2-ethylhexyl) phosphate; Fluorescence quenching method; Densitometry; Tensiometry; Critical aggregation concentration; Aggregation number; Partial specific volume

1. Introduction

The separation of electrically neutral solutes by capillary electrophoresis (CE) usually is not possible because nonionic solutes do not have a charge. Thus, neutral molecules move with the electroosmotic flow (EOF) through the capillary. However, separation of neutral molecules has been achieved by adding ionic surfactants as pseudostationary phases to the running buffer [1]. This mode of CE is commonly referred to as micellar electrokinetic chromatography (MEKC). Various types of surfactants such as anionic [2–9], cationic [10–12], nonionic [13–15], and zwitterionic [15,16] have been used as pseudostationary phases in MEKC for the separation of both ionic and nonionic solutes.

In MEKC, uncharged solutes are separated based on their differential partitioning between the aqueous phase and the pseudostationary phase. The hydrophobic interaction between solutes and the pseudostationary phase usually is the major driving force behind the solute retention in MEKC. A major advantage of MEKC over many separation techniques is the feasibility of changing the chemical composition of the MEKC system by simply rinsing the capillary with the solution of a new pseudostationary phase.

In the present report, two chemically similar surfactants, i.e., sodium di(2-ethylhexyl) sulfosuccinate (DOSS) and sodium di(2-ethylhexyl) phosphate (NaDEHP) (Fig. 1) were used. Both surfactants possess double hydrophobic alkyl chains and negatively charged head groups. In addition,

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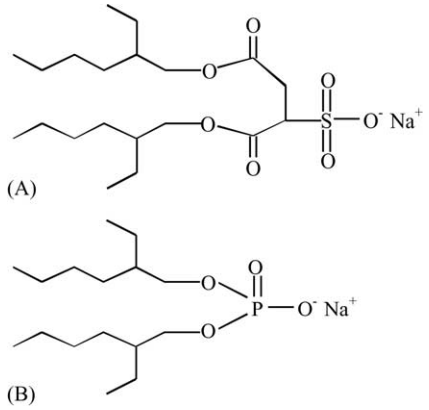

Fig. 1. Chemical structures of (A) sodium di(2-ethylhexyl) sulfosuccinate and (B) sodium di(2-ethylhexyl) phosphate.

NaDEHP has been used extensively as an extraction agent for the separation and purification of a variety of chemicals such as molybdenum isotopes [17], trivalent lanthanides [18], basic and quaternary drugs [19], and albuterol in guinea pig serum [20]. In comparison, DOSS has been used in several studies as a pseudostationary phase in MEKC [3–6], as an ion-pairing agent in reversed phase ion-pair liquid chromatography [21], as an additive to capillary electrochromatography [22] and as an additive to high performance liquid chromatography [23] for separation of hydrophobic solutes.

In the research described here, both DOSS and NaDEHP were characterized using fluorescence quenching, densitometry, and tensiometry techniques to determine their aggregation number, partial specific volume, and critical aggregation concentration. The surfactants were then applied as pseudostationary phases in MEKC for separation of alkyl phenyl ketones. To the best of our knowledge, there is only one report on the successful use of NaDEHP as a pseudostationary phase for the separation of neutral solutes in MEKC [7].

2. Experimental

2.1. Reagents and chemicals

Disodium hydrogen phosphate, alkyl phenyl ketone homologues (Fig. 2), pyrene, and cetylpyridinium chloride were obtained from Aldrich (Milwaukee, WI, USA). Sodium di(2-ethylhexyl) sulfosuccinate and sodium di(2-ethylhexyl) phosphate were purchased from Sigma (St. Louis, MO, USA). All chemicals were used as received. Deionized water of 18 MΩ quality water was used for all aqueous buffer solutions.

2.2. MEKC instrumentation

All MEKC experiments were performed on a Beckman P/ACE model 5510 CE instrument (Fullerton, CA, USA) equipped with a 0–30 kV power supply, a 21-position inlet

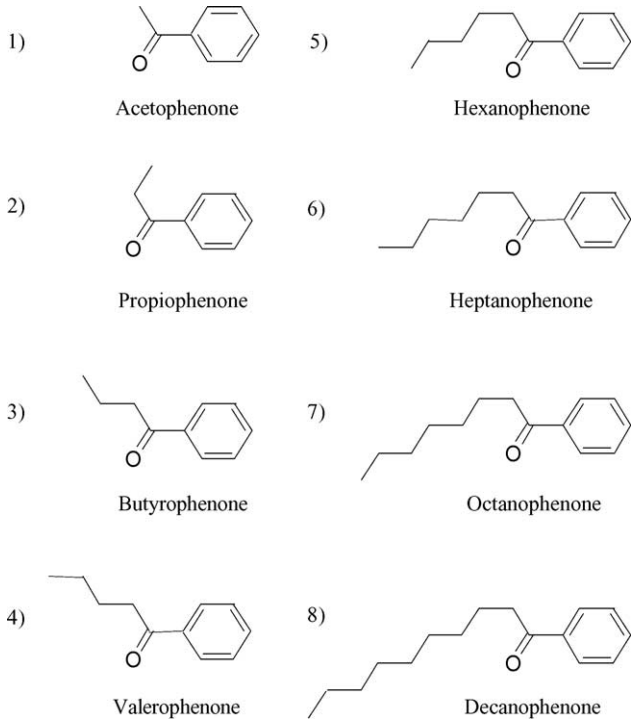

Fig. 2. Chemical structures of the alkyl phenyl ketones.

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 47 cm total length (40 cm to the detector) × 50 μm i.d. × 375 μm o.d.], and software System Gold for system control and data handling. The capillary was thermostated by use of a fluoroorganic fluid. The detector was operated at 254 nm for detection of alkyl phenyl ketones. All experiments were performed at 20 °C. A voltage of +20 kV was applied for all MEKC separations.

2.3. Capillary electrophoresis procedure

All new capillaries were activated by the following washing sequence: 1 M NaOH (30 min) followed with triply deionized water (20 min). Prior to each separation with the same surfactant the capillaries were rinsed with triply deionized water (5 min), 0.1 M NaOH (3 min), and separation buffer (3 min). When the surfactant was changed, the capillaries were 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 0.5 p.s.i. for 2 s. (1 p.s.i. = 6894.76 Pa).

2.4. Preparation of separation buffers and standard solutions

A 100 mM stock solution of phosphate buffer (pH 9.0) was prepared by dissolving an appropriate amount of disodium hydrogenphosphate and refrigerated after each use.

The DOSS and NaDEHP solutions were prepared by first dissolving appropriate amounts of surfactant in 5.0 mL of deionized water. Two milliltres of the 100 mM phosphate stock buffer was then added to this solution and the final volume adjusted to 10.0 mL with deionized water. The final concentrations of phosphate buffer, DOSS, and NaDEHP were 20, 10 and 30 mM, respectively. 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 CE experiments. All stock alkyl phenyl ketone solutions were prepared in methanol at concentrations of 4–7 mM each.

2.5. Characterization of DOSS and NaDEHP

2.5.1. Determination of critical aggregation concentration

The surface tension method was used to estimate the critical aggregation concentration (CAC) of DOSS and NaDEHP surfactants in aqueous solution. A 20 mM stock solution of each of DOSS and NaDEHP surfactants was prepared in deionized water. Ten to fifteen different concentrations ranging from 0.1 to 20.0 mM were prepared from the stock solution and used in the determination of CAC. A Du Nüoy type tensiometer was used for surface tension measurements.

2.5.2. Determination of aggregation number

The aggregation number (N) of the surfactants was determined by a fluorescence quenching method proposed by Turro and Yekta [24], using the following expression:

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

where I_0 and I are the emission intensities in the absence and presence of the quencher, respectively. The parameter $[S_{tot}]$ is the total surfactant concentration and $[Q]$ is the quencher concentration in the surfactant solution. Fluorescence measurements were performed on a Perkin-Elmer fluorescence spectrophotometer (LS 50B model). Pyrene and cetylpyridinium chloride were used as fluorescent probe and quencher, respectively.

A 1 mM stock solution of pyrene was prepared in methanol. Two stock solutions of the quencher with each surfactant were prepared in deionized water, i.e., 2.8 mM cetylpyridinium chloride together with 20 mM DOSS and 2.8 mM cetylpyridinium chloride with 60 mM NaDEHP. A known volume of pyrene stock solution was pipetted into two clean volumetric flasks, and the methanol was evaporated under nitrogen gas. Aqueous surfactant solutions of DOSS and NaDEHP were then added to each volumetric flask. At this step, the concentrations of pyrene, DOSS, and NaDEHP were 2.0×10^{-3}, 20, and 60 mM, respectively (solution 1A). Each surfactant solution (i.e., pyrene + DOSS and pyrene + NaDEHP) was sonicated for 90 min and stored in the dark overnight to equilibrate. Then, solution 1A of each surfactant was divided in half: one half was diluted with deionized water to give a 1.0×10^{-3} mM pyrene and 10 mM DOSS or 30 mM NaDEHP (solution 2A) and the other half was mixed with quencher stock solution to make 0.14 mM cetylpyridinium chloride, 1.0×10^{-3} mM pyrene and 10 mM DOSS or 30 mM NaDEHP (solution 3A). Solution 3A was added to solution 2A in increasing 100 μL increments and allowed to equilibrate for 15 min before collecting fluorescence measurements. The decrease in emission spectra of pyrene was recorded at 393 nm with an excitation at 335 nm after each aliquot of quencher solution (solution 3A) was added, and the logarithm of the intensity ratio I_0/I was plotted against the quencher concentration. The aggregation number is obtained from the slope of the plot of $\ln(I_0/I)$ versus $[Q]$ (i.e., $N = \text{slope} \times [S_{tot}] - \mathrm{CAC}$).

2.5.3. Determination of partial specific volume

The partial specific volume, $\bar{v}$, is defined as an increase in volume upon dissolving 1 g of a dry material (e.g., surfactant) in a large volume of a solvent (e.g., water) when the mass of solvent, temperature, and pressure are held constant. The value of $\bar{v}$ can be determined using the following expression:

$$\frac{1}{\rho} = \bar{v} + W\frac{\partial(1/\rho)}{\partial W} \quad (2)$$

where ρ is the density of the aqueous surfactant solution and W is defined as the weight fraction of solvent, i.e., water in the current study.

Seven different surfactant solutions (i.e., 200, 150, 100, 50, 25, 13, and 6 mg) were prepared in 10 mL deionized water at 20 °C for density measurements. The precision of the temperature-controlled system used in this study was better than ±0.005 °C. Density measurements were performed using a high-precision Anton Paar USA (League City, TX, USA), model DMA 58 digital density meter. The values of $\bar{v}$ for the surfactants used in this study were obtained as the y-intercept of a plot of $1/\rho$ versus W.

2.6. Calculations

The capacity factor, k', of the solutes was measured according to the following equation:

$$k' = \frac{t_R - t_{eo}}{t_{eo}[1 - (t_R/t_{psp})]} \quad (3)$$

where t_R, t_{eo} and t_{psp} are the migration times of a neutral retained solute, the 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_{psp}/t_{eo}.

The apparent electrophoretic mobilities of DOSS and NaDEHP (μ_{app}, $\mathrm{cm^2\ V^{-1}\ s^{-1}}$) were calculated using Eq. (4):

$$\mu_{app} = \frac{l_t l_d}{V t_{psp}} \quad (4)$$

where l_t is the total length of the capillary (cm), l_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 (μ_{eo}, $cm^2\,V^{-1}\,s^{-1}$), of the buffer solution t_{psp} term in Eq. (4) is replaced with t_{eo}. Methanol was used as the EOF marker. The t_{eo} was taken as the first deviation from baseline after injection. The effective electrophoretic mobility (μ_{ep}) of each surfactant system was calculated from their net electrophoretic velocity (v_{net}, $cm\,s^{-1}$) values:

$$v_{net} = v_{ep} - v_{eo} = \frac{l_d}{t_{psp}} - \frac{l_d}{t_{eo}} \quad (5)$$

$$\mu_{ep} = \frac{v_{net} l_t}{V} \quad (6)$$

where v_{ep} and v_{eo} are electrophoretic velocity and electroosmotic velocity of pseudostationary phase, respectively.

The methylene (or hydrophobic) selectivity, α_{CH_2}, was calculated from the antilogarithm of the slope of the regression line of $\log k'$ versus carbon number of alkyl phenyl ketone homologous series.

3. Results and discussion

3.1. Physicochemical properties of pseudostationary phases

The primary structural difference between DOSS and NaDEHP is their head groups; DOSS has a sulfosuccinate head group while NaDEHP has a phosphate head group. Both surfactants have sodium as a counterion and 2-ethylhexyl alkyl chain as their hydrophobic moiety. The physicochemical properties of these two surfactants are compared in Table 1.

3.1.1. Critical aggregation concentrations of DOSS and NaDEHP

The measured surface tension values for DOSS and NaDEHP in water at room temperature were plotted against surfactant concentration. The CAC values were determined as the inflection point of the two straight lines that fit the experimental values before and after the abrupt change of slope (Fig. 3). As seen in Fig. 3, the CAC values for DOSS and NaDEHP are 1.80 and 5.12 mM, respectively.

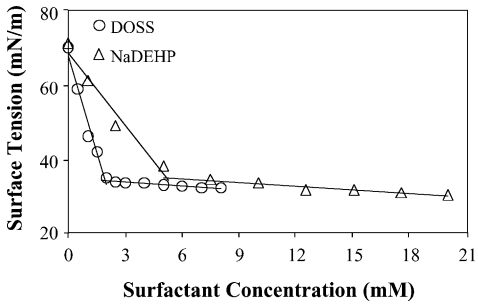

Fig. 3. Variation of the surface tension with the concentration of DOSS (○) and NaDEHP (△) in aqueous solution at room temperature. Legends are shown in the plot.

Table 1
Comparison of physicochemical properties of DOSS and NaDEHP surfactants

Physicochemical property	Pseudostationary phase	
	DOSS	NaDEHP
Molecular formula	$C_{20}H_{37}NaO_7S$	$C_{16}H_{35}NaO_4P$
Aggregation number[a], N	35	74
Molecular mass, M (g/mol)	444.6[b]	345.4[b]
	1.556×10^4[c]	2.556×10^4[c]
Critical aggregation concentration[d], CAC (mM)	1.80	5.12
Partial specific volume[e] (mL/g)	0.837	0.900
Electroosmotic mobility[f,g], μ_{eo} ($10^{-4}\,cm^2\,V^{-1}\,s^{-1}$)	5.46	5.05
Apparent electrophoretic mobility[f,h], μ_{app} ($10^{-4}\,cm^2\,V^{-1}\,s^{-1}$)	0.63	1.42
Effective electrophoretic mobility[f,i], μ_{ep} ($10^{-4}\,cm^2\,V^{-1}\,s^{-1}$)	−4.83	−3.63
Methylene group selectivity[f,j], α_{CH_2}	3.43	2.78
Migration-time window[f], t_{psp}/t_{eo}	8.71	3.55

[a] Determined in deionized water by fluorescence quenching method.
[b] Molecular weight of one mole surfactant.
[c] Molecular weight calculated from aggregation number.
[d] Determined in deionized water by surface tension measurement.
[e] Determined from density measurement.
[f] Data were collected with 47 cm (40 cm effective length) × 50 μm i.d. capillary with an applied voltage of +20 kV using a 20 mM phosphate buffer at pH of 9.0, final surfactant concentration was 10 mM (∼6 times CAC) DOSS and 30 mM (∼6 times CAC) NaDEHP.
[g] Calculated using Eq. (4), t_{psp} was replaced with t_{eo}.
[h] Calculated using Eq. (4).
[i] Calculated using Eq. (6).
[j] Calculated from the antilogarithm of the slope of the regression line of $\log k'$ vs. carbon number of alkyl phenyl ketones (C_8–C_{14}).

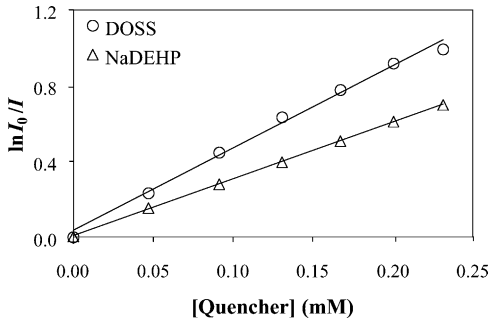

Fig. 4. Determination of aggregation number of DOSS (◯) and NaDEHP (△) using fluorescence quenching method. Legends are shown in the plot.

3.1.2. Aggregation numbers

Fluorescence spectra of pyrene were recorded at several quencher concentrations. An increase in quencher concentration decreases the fluorescence intensity of pyrene molecule in aqueous surfactant solution. The aggregation number, *N*, of each surfactant was obtained from the slope of ln (I_0/I) versus [*Q*] plot (Fig. 4). Experimental values of *N* for the surfactants are presented in Table 1. Aggregation numbers of 35 and 74 were determined for DOSS and NaDEHP, respectively. The relatively bulkier sulfosuccinate head group of DOSS might be the major factor in the lower aggregation number for this surfactant.

3.1.3. Partial specific volumes

Since it is difficult to measure the exact volume of a particle (e.g., micelle), partial specific volume, $\overline{v}$, is a technique that is used more frequently. The $\overline{v}$ of the two surfactants were obtained as the y-intercepts of plots of the $1/\rho$ versus *W* (Fig. 5). As seen in Table 1, the $\overline{v}$ value of DOSS is lower (0.837) than that of NaDEHP (0.900), which indicates that DOSS has a more compact structure whereas NaDEHP has a relatively flexible structure.

3.1.4. Electroosmotic, apparent, and effective electrophoretic mobilities

The DOSS system has a slightly higher μ_{eo} value (5.46×10^{-4} cm^2 V^{-1} s^{-1}) than that of the NaDEHP system (5.05×10^{-4} cm^2 V^{-1} s^{-1}) (Table 1). The difference in μ_{eo} values for each surfactant system is likely due to the difference in Zeta potentials of the capillary wall, of pseudostationary phase, or the difference in the viscosity of the separation buffer.

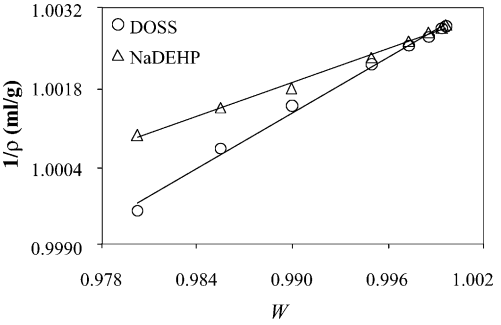

Fig. 5. Plot of $1/\rho$ as a function of *W* for DOSS (◯) and NaDEHP (△) in aqueous solution at room temperature. Legends are shown in the plot.

The various negative values of μ_{ep} are related to the net charge and the size of the aggregates as well as the viscosity of the buffer solution as shown in Eq. (7):

$$\mu_{ep} = \frac{q}{6\pi\eta r} \tag{7}$$

where *q* is the charge on the particle (surfactant aggregate), η is the viscosity of the buffer solution, and *r* is the Stokes′ radius of the surfactant aggregate. According to Eq. (7) and assuming the viscosity of both surfactant solutions is constant, it is fairly evident that the DOSS system has a larger *q*/*r* value than the NaDEHP system (Table 1, μ_{ep} values). The μ_{app} of the DOSS system (0.63×10^{-4} cm^2 V^{-1} s^{-1}) is smaller than that of NaDEHP (1.42×10^{-4} cm^2 V^{-1} s^{-1}). The DOSS system provides a wider separation window ($t_{psp}/t_{eo} = 8.71$) than the NaDEHP system ($t_{psp}/t_{eo} = 3.55$). This is largely due to the relatively lower effective mobility of the DOSS system (-4.83×10^{-4} cm^2 V^{-1} s^{-1}) as compared to that of the NaDEHP system (-3.63×10^{-4} cm^2 V^{-1} s^{-1}).

3.1.5. Methylene-group selectivity

Methylene selectivity (α_{CH_2}) is the average chromatographic selectivity between adjacent analytes in a homologous series. It is a measure of the polarity of a pseudostationary phase, where lower α_{CH_2} values indicate more polar character. The methylene selectivity of each pseudostationary phase was calculated from the antilogarithm of the slope of the regression line of log *k*′ versus carbon number of alkyl phenyl ketone homologous series. Fig. 6 shows the plots of log *k*′ values of alkyl phenyl ketones in DOSS and NaDEHP surfactants versus carbon number of alkyl phenyl ketones. The DOSS surfactant system provides a more hydrophobic environment ($\alpha_{CH_2} = 3.43$) than the NaDEHP surfactant system ($\alpha_{CH_2} = 2.78$).

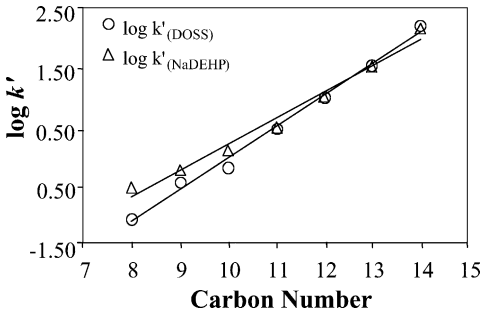

Fig. 6. Linear relationship between log *k*′ vs. carbon number of alkyl phenyl ketone homologous series using DOSS (◯) and NaDEHP (△) systems as pseudostationary phases. MEKC conditions: 20 mM phosphate buffer (pH 9.0); applied voltage: +20 kV; separation temperature: 20 °C; UV detection at 254 nm; separation capillary dimensions: 47 cm (40 cm to the detector) × 50 μm i.d. × 375 μm o.d. Alkyl phenyl ketones are: acetophenone (C_8), propiophenone (C_9), butyrophenone (C_{10}), valerophenone (C_{11}), hexanophenone (C_{12}), heptanophenone (C_{13}), and octanophenone (C_{14}).

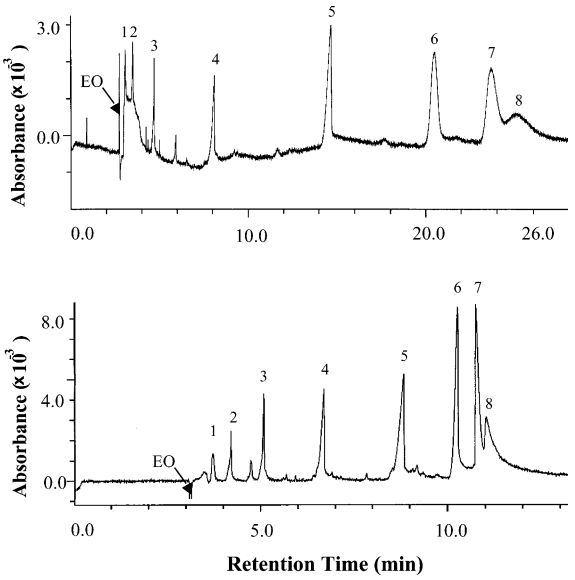

Fig. 7. Electrokinetic separation of alkyl phenyl ketone homologous series using 10 mM DOSS (top) and 30 mM NaDEHP (bottom). MEKC conditions same as Fig. 6. Peak identifications: (1) acetophenone, (2) propiophenone, (3) butyrophenone, (4) valerophenone, (5) hexanophenone, (6) heptanophenone, (7) octanophenone, and (8) decanophenone.

3.1.6. Application of DOSS and NaDEHP as pseudostationary phases in MEKC

The retention behaviour of eight alkyl phenyl ketones (i.e., acetophenone to decanophenone) was examined using DOSS and NaDEHP as pseudostationary phases in MEKC (Fig. 7). Solute interactions with the pseudostationary phases occur through a variety of mechanisms such as surface adsorption, coaggregation or partitioning into the hydrophobic core of pseudostationary phases. Due to these different mechanisms, the retention factors of the test solutes in each pseudostationary phase are not identical. As seen in Fig. 8, the more hydrophilic analytes (i.e., analytes 1–3) interact more strongly with the hydrophilic NaDEHP surfactant relative to the hydrophobic DOSS surfactant. In contrast, the hydrophobic analytes (i.e., analytes 5–7) interact more strongly with the relatively hydrophobic DOSS surfactant than the hydrophilic NaDEHP surfactant.

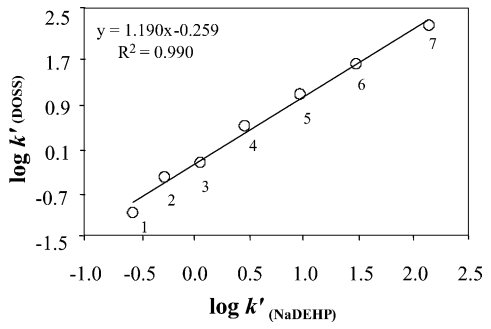

Fig. 8. Capacity factor comparison of alkyl phenyl ketones using DOSS and NaDEHP.

4. Conclusion

Two water-soluble anionic surfactants, i.e., DOSS and NaDEHP, with double alkyl chains were characterized using a number of analytical techniques such as fluorescence quenching, densitometry and tensiometry to determine aggregation numbers, partial specific volumes, and critical aggregation concentrations. The aggregation number of NaDEHP was found to be more than two-fold higher than that of DOSS. The partial specific volume of NaDEHP and DOSS was found to be 0.900 and 0.837 mL/g, respectively. The critical aggregation concentration was 5.12 and 1.80 mM for NaDEHP and DOSS, respectively. These two surfactants were then applied as pseudostationary phases in micellar electrokinetic chromatography for the separation of alkyl phenyl ketones. The DOSS surfactant provided a wider separation window than NaDEHP under the experimental conditions studied. Hydrophobic analytes tend to interact more strongly with DOSS surfactant due to its higher hydrophobic character, whereas hydrophilic analytes interact more with NaDEHP.

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