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# Anionic–zwitterionic mixed micelles in micellar electrokinetic chromatography: sodium dodecyl sulfate–N-dodecyl-N,N-dimethylammonium-3-propane-1-sulfonic acid

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**Abstract**

A zwitterionic surfactant, N-dodecyl-N,N-dimethylammonium-3-propane-1-sulfonic acid (SB-12), was used in combination with an anionic surfactant, sodium dodecyl sulfate (SDS), to form a novel pseudostationary phase for use in micellar electrokinetic chromatography. This mixed micellar system was characterized in terms of analyte retention, selectivity, efficiency, elution range, and resolution; and compared to results obtained using only SDS. A typically used SDS concentration, 20 m*M*, was chosen as a reference to which comparisons could be drawn. With 20 m*M* SDS, the optimum concentration range of 10–20 m*M* SB-12 provided efficiencies that were 2–4 times greater than with SDS alone, with minimal ( $< 15\%$) changes in the elution range and electroosmotic flow. The addition of 40 and 60 m*M* SB-12 also resulted in efficiencies on average of 600 000–800 000 theoretical plates/m, but at a significant reduction in the elution range and peak capacity. Retention factors ($k'$) for the various neutral analytes increased by 20% with addition of 10 m*M* SB-12 and by approximately 60% with addition of 40 and 60 m*M* SB-12, while operating currents remained constant as SB-12 was added. Geometrical isomers *p*-nitrotoluene and *m*-nitrotoluene, that co-eluted with 20 m*M* SDS, were baseline resolved with the addition of 10 m*M* SB-12; in addition, methylene selectivity was greatest at this composition. No capillary wall interactions or coating effects were observed with the SDS–SB-12 mixed micellar system, in contrast to previously studied anionic–non-ionic mixed micellar system, SDS–Brij 35. Consequently, migration times were very repeatable ( $\leq 1.2\%$ R.S.D.).

## 1. Introduction

Capillary electrophoresis (CE) allows for the high-resolution separation of charged analytes and numerous applications involving the use of CE have appeared in the literature. The introduction of micellar electrokinetic capillary chromatography MEKC) by Terabe *et al.* in 1984 [1] allowed for the separation of neutral analytes which had not possible with CE alone. To date MEKC has been utilized to separate a diverse range of analytes such as gun shot residues [2,3], phenylthiohydantoin-amino acids [4,5], nucleic acid constituents [6–9], $\beta$-blockers [10], water- and fat-soluble vitamins [11,12], and herbicides [13].

The addition of surfactant(s) above their critical micelle concentration (CMC) provides a pseudostationary phase that neutral analytes can differentially interact with. Unlike CE in free solution, where the separation mechanism is one based on differences in the electrophoretic mo-

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bilities, $\mu_{ep}$, of the charged analytes, the separation mechanism for neutral analytes in MEKC is one based on differential partitioning between the micellar pseudostationary phase and the aqueous phase.

One of the challenges in MEKC is the improvement of resolution via efficiency or selectivity enhancement, without significantly increasing analysis time, operating currents, or any other effects that could be detrimental in achieving the optimum MEKC separation. Most of the additives that have been utilized in MEKC to obtain better separations unfortunately come at the expense of increased analysis time, decreased efficiencies, or both. For example, the addition of organic modifiers such as acetonitrile to the separation medium has been shown to increase the elution range but at a cost of increased analysis time, higher operating currents, and lower separation efficiencies [14–16]. The addition of glucose in MEKC facilitated the separation of nine nucleosides, but also at a cost of increased analysis time [17]. Although these various additives have advantages, their use in MEKC is not without some drawbacks.

Numerous surfactants are commercially available and could be utilized as pseudostationary phases in MEKC. To date the surfactant of choice, however, has been sodium dodecyl sulfate (SDS) as evidenced by the large number of articles published in MEKC with an SDS-based micellar system. It seems logical then, that the use of different surfactants or even combinations of surfactants to form mixed micelles to improve separations in MEKC should be investigated. Moderate changes in selectivity variation due to changes in surfactant identity has been reported [9]. A novel pseudostationary, sodium 10-undecylenate oligomerized to form a micelle-like structure, which permitted the use of high percentages of organic solvent has also been reported [18]. Recently, Nielsen and Foley [19] investigated the effects of surfactant counter ion on separations in MEKC. In a comparison between $Mg^{2+}$ and $Na^{+}$ counter ions of dodecyl sulfate, the $Mg(DS)_2$ micellar system provided much higher methylene and functional group selectivities than the similar SDS-based separations. In addition, retention factors were between 1.5 and 2.5 times larger for the analytes studied with $Mg(DS)_2$. These increases in selectivity and retention resulted in much better resolution of the analytes studied. These examples serve to illustrate the need to further investigate and characterize other novel surfactant systems that could possibly provide improved separations in MEKC.

One specific area that has received little attention is the use of mixed micelles as a pseudostationary phase in MEKC. Mixed micelles are formed when two or more surfactants are used together provided they are compatible [20]. The use of mixed micelles in MEKC has been limited to anionic–non-ionic micellar systems such as SDS–Brij 35 [5,13,16,21,22]. The studies performed with the SDS–Brij 35 mixed micellar systems showed that significant selectivity changes and consequently improved separations could be generated through the use of such systems over conventional SDS micellar systems. Although these initial studies clearly showed the advantages of using a mixed micellar pseudostationary phase, one of the problems with the SDS–Brij 35 pseudostationary phase however, is the capillary wall coating effects [23] of Brij 35 that tend to significantly alter solute retention if many runs are done in sequence [16]. With each separation, the capillary surface is coated a little more with Brij 35, resulting in slower and slower electroosmotic flows. It is the slower electroosmotic flows that cause analyte migration times to increase or what we term here as "electroosmotic drift".

Zwitterionic surfactants have been used even less in CE and MEKC. Swedburg [24] used a the zwitterionic surfactant, 3-[3-(chloroamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), to achieve selectivity enhancements for the separation of some heptapeptides. An apparent MEKC mechanism was proposed. More recently, the zwitterionic surfactant 3-(N,N-dimethylhexadecylammonium)propane-sulfonate (PAPS) was added to the running buffer to aid in the separation of polymyxins [25]. Again, an MEKC separation mechanism was proposed.

The use of zwitterionic surfactants in the

MEKC mode of separation shows considerable promise. To date, however, there have been no reports of zwitterionic surfactants used in combination with either anionic or cationic surfactants to form mixed micelles. The use of such micellar systems could offset the dynamic modification of the silica capillary surface that surfactants like PAPS have been shown to do [25]. These types of surface modifications can lead to "electroosmotic drift" mentioned previously.

To the best of our knowledge, the present study is the first to examine the use of anionic–zwitterionic mixed micelles in MEKC. Here we employ the zwitterionic surfactant, N-dodecyl-N,N-dimethylammonium-3-propane-1-sulfonic acid (SB-12), in combination with the anionic surfactant, SDS, to form a novel pseudostationary phase. This mixed micellar system was investigated in order to examine what effects this type of pseudostationary phase would have on analyte efficiency, retention, elution range, and selectivity, the chromatographic parameters that affect resolution in MEKC. In addition, the repeatability of separations using the SB-12–SDS system was also measured since this was a problem with the SDS–Brij 35 mixed micellar system. Future reports with this mixed micellar system will examine temperature effects as well as the effects of various organic modifiers on the chromatographic parameters that influence analyte resolution in MEKC. Although from our chromatographic perspective organic modifiers have proven to be largely disadvantageous with pure SDS micellar systems (above), we do not necessarily anticipate these disadvantages in mixed micellar systems because of significant differences in the two types of micellar systems.

## 2. Experimental

### 2.1. Apparatus

A Waters Quanta 4000 capillary electrophoresis system (Millipore, Waters Chromatography Division, Milford, MA, USA) equipped with fixed-wavelength UV detection at 254 nm was employed for all the separations performed in this study. All of the test analytes were detectable at this wavelength. MEKC was performed in a 30 cm (injection to detection) × 50 μm I.D. × 370 μm O.D. fused-silica capillary tube (Polymicro Technologies, Tucson, AZ, USA). The total capillary length was 37.5 cm. Injections were made hydrostatically for 3 s. The applied voltage was 20 kV and operating currents were less than 35 μA unless otherwise noted in the text. The data were collected at a rate of 10 points/s and analyzed on a Macintosh IIci computer (Apple, Cupertino, CA, USA) using a MacLab 4 channel ADC with the appropriate vendor software (ADInstruments, Milford, MA, USA). All experiments were done at ambient temperature (*ca.* 25°C).

### 2.2. Materials

All the neutral test analytes were purchased from Aldrich (Milwaukee, WI, USA) unless otherwise noted. The neutral test mixture consisted of benzyl alcohol (EM Science, Gibbstown, NJ, USA), nitrobenzene, anisole, *p*-nitrotoluene, *m*-nitrotoluene, benzophenone, biphenyl (MCB Reagents, Cincinnati, OH, USA), and decanophenone. A homologous series of alkylphenones were purchased as a kit from Aldrich. The novel pseudostationary phase was made up of SB-12, purity 98% (Aldrich) employed in combination with SDS which was purchased from Sigma (St. Louis, MO, USA). Both surfactants were used as received. The concentration of SDS was 20 m*M* for all the MEKC separations. The SB-12 concentrations ranged from 5, 10, 20, 40 to 60 m*M*. Stock buffer solutions were prepared with $NaH_2PO_4 \cdot H_2O$ and sodium hydroxide to give a 100 m*M* phosphate buffer at pH 7.0. A phosphate buffer concentration of 10 m*M* was used in all the experiments. The mixed micellar solutions were made by weighing appropriate amounts of SDS and SB-12 and diluting with the stock buffer solution and distilled water in a 100-ml volumetric flask to obtain the desired concentrations. All the micellar buffer solutions were filtered through 0.20-μm membrane filters obtained from Alltech (Deerfield, IL, USA) and degassed

before use. HPLC-grade distilled water used in the makeup of the micellar buffer solutions was obtained from J.T. Baker (Phillipsburg, NJ, USA). Sample solutions were made up of 25% acetonitrile and 75% running buffer with solute concentrations at or below 1.5 mg $ml^{-1}$.

### 2.3. Methods

The capillary was activated using a modification of a previously described procedure [26]. The capillary was first rinsed with 1 *M* KOH for 20 min followed by subsequent rinses of 0.1 *M* KOH and distilled water for 20 min each. A final 20-min rinse was performed with the operating buffer before the capillary was used. Purges with the operating buffer were done after each run for 5 min using a vacuum of *ca.* 42 cmHg (1 cmHg = 1333.22 Pa) at the detector reservoir.

Electroosmotic velocities were measured using a method previously published [27]. The $t_{mc}$ values, which represent the elution time of the pseudostationary phase for each separation, were measured using decanophenone and confirmed with the iterative computation method developed by Bushey and Jorgenson [28]. Equations used in the calculation of separation efficiencies, retention factors, selectivities, and resolution are discussed in section 2.4 of the present paper.

All runs were done in sets of five so as to monitor any wall adsorption effects that may occur. These types of effects have been seen with other mixed micellar systems such as SDS–Brij 35. The result of such wall interactions or capillary coating is the non-repeatability of the runs, clearly a disadvantage. The SB-12–SDS micellar system used here showed no signs of such wall effects as all runs were very repeatable. In addition, the set of five runs at each mixed micellar concentration were done in sequence with increasing amounts of SB-12 and then repeated, so even after 20–30 runs no wall effects were observed. This is further substantiated by the repeatability of the migration times for the test analytes ($\leq$1.2% R.S.D.). If any capillary coating was occurring, we would also have observed increased band broadening and a decrease in column efficiency which we did not see.

### 2.4. Calculations

Retention factors for all the analytes studied were calculated using Eq. 1,

$$k' = \frac{t_R - t_0}{t_0\left[1 - \frac{t_R}{t_{mc}}\right]} \quad (1)$$

where $t_R$, $t_0$ and $t_{mc}$ are the migration times of the analyte of interest, an unretained analyte and the micelle, respectively.

The resolution for the two geometrical isomers, *p*-nitrotoluene and *m*-nitrotoluene, was calculated using the fundamental resolution equation in MEKC,

$$R_s = \left[\frac{\sqrt{N}}{4}\right]\left[\frac{\alpha - 1}{\alpha}\right]\left[\frac{k_2'}{1 + k_2'}\right]\left[\frac{1 - \frac{t_0}{t_{mc}}}{1 + \left[\frac{t_0}{t_{mc}}\right]k_1'}\right] \quad (2)$$

where $N$ is the number of theoretical plates and $\alpha$, the selectivity, is $k_2'/k_1'$ such that $\alpha$ is always greater than 1. It is evident from Eq. 2 that improvements in resolution can be achieved by (i) increasing efficiency, (ii) enhancing $\alpha$ or (iii) increasing $t_{mc}/t_0$.

The efficiencies of all analytes were calculated using the Foley–Dorsey equation [29],

$$N = \frac{41.7\left[\frac{t_R}{W_{0.1}}\right]^2}{1.25 + \frac{B}{A}} \quad (3)$$

where $W_{0.1}$ is the width of the peak at 10% of the peak height and $B/A$ is the asymmetry factor of the peak. This equation was used to calculate peak efficiencies because it takes into account asymmetric peak shapes [30] and does not incorrectly assume a Gaussian peak profile.

## 3. Results and discussion

### 3.1. General advantages of anionic–zwitterionic and anionic–non-ionic mixed micelles

The use of mixed micellar systems involving charged and net zero charge surfactants like the anionic SDS and the zwitterionic SB-12 offers some advantages in MEKC. First, if the concentration of charged surfactant is held constant, the concentration of net zero charge (NZC) surfactant can be varied without an increase in operating currents. This is possible because the NZC surfactant (non-ionic or zwitterionic) does not contribute to an increase in current upon its addition. High operating currents can lead to undesirable Joule heating effects, which in turn can lead to poor separations. Equally as important, the phase ratio can also be increased in this fashion in order to optimize analyte retention [5] and increase the solubility of analytes in the running buffer. The SDS–Brij 35 and SDS–SB-12 mixed micellar systems have both been shown to increase separation efficiencies by at least a factor of 2 over SDS. This may or may not be a general characteristic of all mixed micellar systems, but at least for these two mixed micellar systems studied to date, the enhancement in separation efficiencies were significant. The addition of NZC surfactants to form mixed micelles does not increase analysis times to the extent that organic modifiers have been shown to do. If one views the addition of NZC surfactants in the same light as the addition of organic modifiers like acetonitrile and methanol, NZC surfactants have the advantage in that they can be used to alter analyte selectivity without the loss in efficiencies or the substantial increases in analysis times that are seen with organic modifiers.

### 3.2. Specific advantages of the SDS–SB-12 mixed micellar system

There are numerous zwitterionic surfactants that are commercially available. SB-12 was selected for several reasons. First, SB-12 is pH independent and therefore in its zwitterionic form at all pH values [20]. This eliminates the need to operate at pH values that would result in either too high or too low electroosmotic flow velocities. In addition, if charged analytes are present, one could operate at the pH necessary to keep the analyte either charged or to render it neutral without being constrained to a specific pH range in order to maintain the SB-12 in its zwitterionic state. Second, as seen in Table 1, the CMC, aggregation number and Krafft point of both SDS and SB-12 are similar, especially in comparison to non-ionic NZC surfactants such as the Brij series, for which CMC values are approximately 20 to 900 times lower than SDS [20]. We expect the CMC of the mixed SDS–SB-12 system to be somewhere in between that of pure SDS and pure SB-12 [20]. Importantly, the surfactant concentrations employed in this study were well above the CMC values of either pure surfactant. The aggregation number is simply the number of surfactant monomers necessary to form a micelle. The Krafft point is defined as the temperature at which the solubility of an ionic surfactant is equal to its CMC. Below this temperature the surfactant will precipitate from solution [20]. Since the Krafft points of both

Table 1
Surfactants and their CMCs, aggregation numbers and Krafft point values [34]

| Surfactant | CMC (*M*) | Aggregation number | Krafft point (°C) |
|---|---|---|---|
| Anionic: SDS | | | |
| $[CH_3(CH_2)_{11}OSO_3^-Na^+]$ | 0.0081 | 62 | 9 |
| Zwitterionic: SB-12 | | | |
| $[CH_3(CH_2)_{11}N^+(CH_3)_2(CH_2)_3SO_3^-]$ | 0.003 | 55 | <0 |

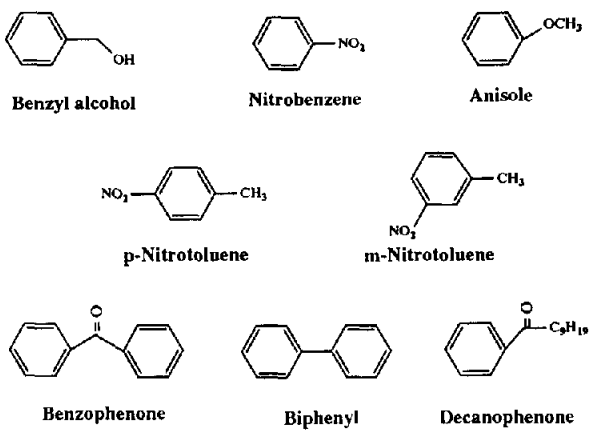


Fig. 1. Structures of neutral test analytes used to characterize the SB-12–SDS mixed micellar system.

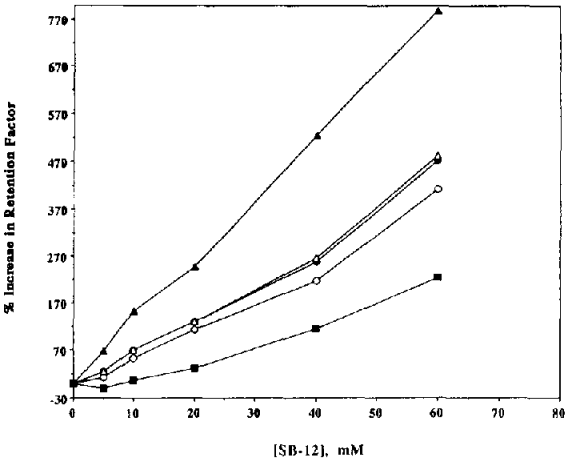


Fig. 2. The % increase in retention factor for selected neutral analytes as a function of increasing SB-12 concentrations. Note: the % increases in retention factor shown, are the increases in retention factor relative to that obtained with 20 m*M* SDS (no SB-12). ○ = Benzyl alcohol; ● = nitrobenzene; △ = anisole; ■ = benzophenone; ▲ = biphenyl.

SDS and SB-12 are less than a typical room temperature of 25°C, use of this mixed micellar system does not require operating at an elevated temperature. This is advantageous from the standpoint of commercial electrophoresis units, since many are not equipped with complete capillary, sample, and exit reservoir thermostating. Finally, we did not expect to see any capillary coating effects with the SDS–SB-12 system.

### *3.3. Retention behavior of analytes with SB-12–SDS*

One of the objectives of this study was to investigate the effect of SB-12 concentrations on the retention of neutral analytes. The neutral analytes chosen for this investigation are shown in Fig. 1. These compounds were selected with respect to their (i) differing range of hydrophobicity, (ii) ease of detection and (iii) solubility in the buffer system employed. Some consideration was also given to variation of the analyte functional groups. Table 2 shows the retention factors ($k'$) for some of the neutral analytes. As can be seen, the $k'$ values for all the probe analytes increased as SB-12 was added. The % increases in $k'$, also shown in Fig. 2, were done with respect to separations achieved in which no SB-12 was used.

Table 2
Retention factors for various neutral analytes

| [SB-12] (m*M*) | $k'$ | | | | |
|---|---|---|---|---|---|
| | Benzyl alcohol | Nitrobenzene | Anisole | Benzophenone | Biphenyl |
| 0.0 | 0.17 | 0.40 | 0.50 | 6.51 | 11.25 |
| 5.0 | 0.19 | 0.50 | 0.62 | 5.82 | 18.78 |
| 10.0 | 0.26 | 0.68 | 0.85 | 6.95 | 28.32 |
| 20.0 | 0.36 | 0.92 | 1.15 | 8.57 | 39.11 |
| 40.0 | 0.54 | 1.43 | 1.83 | 13.96 | 70.33 |
| 60.0 | 0.87 | 2.29 | 2.91 | 21.11 | 100.07 |

All runs were done with [SB-12] in combination with 20 m*M* SDS.

Another equation which can be used to calculate $k'$ is given below.

$$k' = P_{wm}\beta \quad (4)$$

Retention factors in MEKC are therefore governed by the water–micelle partition coefficient ($P_{wm}$) and phase ratio ($\beta$). The increases in retention factor seen for the neutral analytes with the SB-12–SDS micellar system are in part due to either an increasing $P_{wm}$ or $\beta$, depending on the nature of the analyte. If this was simply due to an increasing phase ratio effect, then the increase in retention factor should be proportional for all the neutral analytes. With this in mind, the increase in retention factor for benzyl alcohol, nitrobenzene and anisole is fairly proportional (see Fig. 2) and therefore indicative of a phase ratio effect. However, for the analytes benzophenone and biphenyl, retention factors increased disproportionately, indicating a changing water–micelle partition coefficient.

The retention behavior of the analytes can be generalized in that the more hydrophilic analytes experience a phase ratio effect, while the more hydrophobic analytes are subject to a changing water–micelle partition coefficient. This generalization makes sense if one thinks about the loci of analyte solubilization. The more hydrophilic analytes do not penetrate as deeply into the micellar core as the hydrophobic analytes and thus will experience more surface interactions with the micelle. The addition of SB-12, which has a $C_{12}$ hydrocarbon chain similar to SDS, will leave the micellar core with roughly the same hydrophobic character. The zwitterionic charged headgroups of SB-12 will be predominantly present on the surface of the micelle, thus increasing the concentration of SB-12 will affect the surface chemistry of the micelle much more than interior of the micelle. Thus, the retention of hydrophilic analytes will be affected to a greater extent by an increasing phase ratio because they predominantly experience surface interactions with the micelle.

Hydrophobic analytes like biphenyl on the other hand, will partition deeper into the micelle. According to the two state model of solute solubilization, a solute can be in a "dissolved" state in the micellar core or in an "adsorbed" state at the micelle–water interface [31]. If the amount of solute in the "dissolved" or "adsorbed" states increases, then the water–micelle partition coefficient will also increase. One way to increase the amount of analyte in the "dissolved" state is to increase the micelle radius which would result in a proportionally lower Laplacian pressure and increased partitioning into the core of the micelle [19]. In mixed micelles made up of SB-12 and lithium dodecyl sulfate (LDS), the micelle radius was found to increase as the amount of SB-12 increased [32]. Even though we used SDS in our study, the change in the counter ion of the anionic surfactant should not reverse the trend of increasing micelle radius with the addition of SB-12. As the radius of the SDS–SB-12 micelle increases, the amount of hydrophobic analyte in the "dissolved" state increases and so does the water–micelle partition coefficient (more for some analytes than others).

### 3.4. Methylene selectivity ($\alpha_{CH_2}$)

A homologous series of alkylphenones was used to calculate the hydrophobic or methylene selectivity ($\alpha_{CH2}$). Methylene selectivity can be measured from a plot of log $k'$ *vs.* carbon number ($n_c$) or by comparing the ratio ($k'_2/k'_1$) for pairs of homologues that only differ by one methylene group. Measurement of methylene selectivity provides useful information concerning the solvation properties of the aqueous or micellar phases. Little variation in methylene selectivity values would indicate little variation in the solvation properties or polarity of the pseudostationary or aqueous phase.

Table 3 lists the methylene selectivities for the various concentrations of SB-12 with 20 m*M* SDS. All $\alpha_{CH_2}$ values were calculated by plotting log $k'$ *vs.* carbon number and using the linear model equation [33] in the form log $k' = (\log \alpha_{CH_2})n_c + \log \beta$, where the base 10 logarithm of the methylene selectivity is the slope of the line. Fig. 3 shows log $k'$ *vs.* $n_c$ for the alkylphenones at the various SB-12–SDS concentrations. There

Table 3
Dependence of methylene selectivity on micelle composition

| [SB-12]–20 m*M* SDS | Methylene selectivity | $r^2$ |
|---|---|---|
| 0 | 2.50 | 0.996 |
| 5 | 2.62 | 0.993 |
| 10 | 2.68 | 0.991 |
| 20 | 2.55 | 0.995 |
| 40 | 2.46 | 0.998 |
| 60 | 2.41 | 0.998 |

A homologous series of alkylphenones was used.

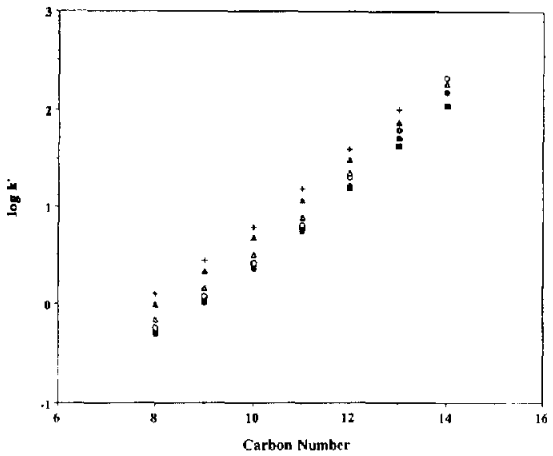


Fig. 3. Log *k'* *vs.* carbon number for the homologous series of alkylphenones. Concentrations of SB-12: ■ = 0 m*M*; ● = 5 m*M*; ○ = 10 m*M*; △ = 20 m*M*; ▲ = 40 m*M*; + = 60 m*M*. SDS concentration: 20 m*M*. Other conditions: applied voltage: 20 kV; operating currents: < 35 μA; capillary length: 30 cm (injection to detection); 3 s hydrostatic injection; temperature: ambient; 10 m*M* phosphate buffer: pH 7.0.

is a slight curvature in the plots but the $r^2$ values were sufficiently high ( > 0.99) so that use of the linear model equation was acceptable. The greatest methylene selectivity occurred when the pseudostationary phase consisted of 10 m*M* SB-12 with 20 m*M* SDS. This is the same concentration range of SB-12 that was found to give the best resolution (see section 3.7). The 5–7% increase in methylene selectivity with the addition of 10–20 m*M* SB-12 in comparison with no SB-12 added, implies that the SB-12–SDS mixed micelles at the specified proportions are slightly less polar than SDS micelles. However, as SB-12 concentration increases to 40 and 60 m*M*, the polarity of the mixed micelles appears to increase to the level of the SDS micelles.

### 3.5. Adjacent analyte selectivity

The variations in selectivity due to different functional groups were calculated using the ratio of retention factors for adjacent pairs of analytes. The neutral test analytes were used in order of increasing retention as follows: benzyl alcohol, nitrobenzene, anisole, *p*-nitrotoluene, *m*-nitrotoluene, benzophenone and biphenyl. Table 4 presents the selectivities for all adjacent pairs of analytes. Fig. 4 depicts the trends in selectivity as a result of adding SB-12. The first four pairs show little variation in selectivity as the concentration of SB-12 is increased. However, for pairs Bz-mNt and B-Bz, the selectivity changes upon the addition of SB-12. The selectivity for pair Bz-mNt decreases by approximately 25% with addition of only 5 m*M* SB-12 and

Table 4
Adjacent analyte selectivity

| [SB-12] (m*M*) | Nb-BA | A-Nb | pNt-A | mNt-pNt | Bz-mNt | B-Bz |
|---|---|---|---|---|---|---|
| 0 | 2.39 | 1.26 | 2.11 | 1.04 | 5.89 | 1.73 |
| 5 | 2.68 | 1.25 | 1.97 | 1.06 | 4.45 | 3.23 |
| 10 | 2.58 | 1.26 | 1.88 | 1.07 | 4.08 | 4.08 |
| 20 | 2.54 | 1.26 | 1.81 | 1.08 | 3.81 | 4.56 |
| 40 | 2.64 | 1.27 | 1.79 | 1.10 | 3.91 | 5.04 |
| 60 | 2.64 | 1.27 | 1.77 | 1.10 | 3.74 | 4.74 |

Solute pair identification: Nb-BA = Nitrobenzene–benzyl alcohol; A-Nb = anisole–nitrobenzene; pNt-A = *p*-nitrotoluene–anisole; mNt-pNt = *m*-nitrotoluene–*p*-nitrotoluene; Bz-mNt = benzophenone–*m*-nitrotoluene; B-Bz = biphenyl–benzophenone.

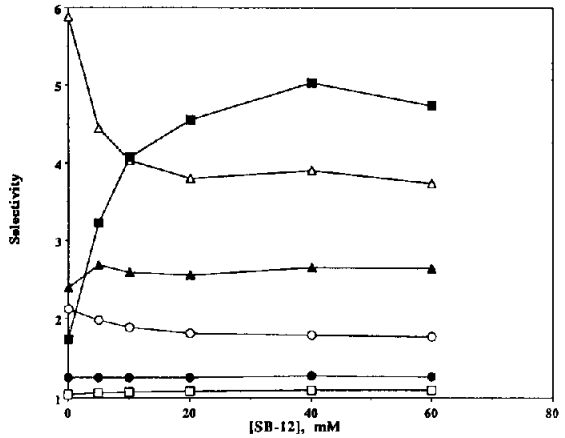


Fig. 4. Variation in selectivity for adjacent pairs of the neutral test analytes as function of [SB-12] added. The concentration of SDS was 20 m*M*. Solute pairs: ▲ = Nitrobenzene–benzyl alcohol; ● = anisole–nitrobenzene; ○ = *p*-nitrotoluene–anisole; □ = *m*-nitrotoluene–*p*-nitrotoluene; △ = benzophenone–*m*-nitrotoluene; ■ = biphenyl–benzophenone. Conditions as in Fig. 3 (see also Experimental section).

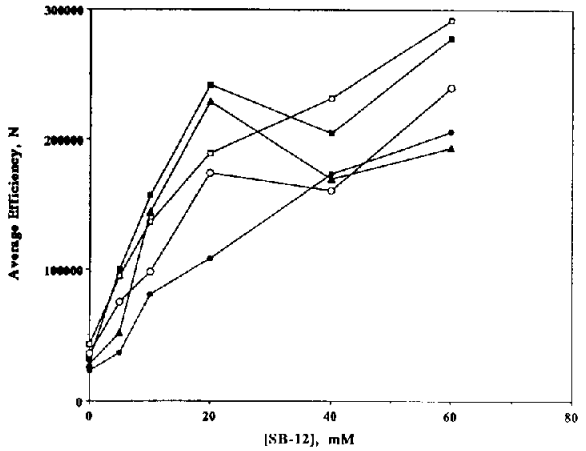


Fig. 5. Effect of micellar composition on efficiency for the alkylphenones (average of five runs). The concentration of SDS was always 20 m*M*. Other conditions as in Fig. 3. ● = Acetophenone; □ = propiophenone; ■ = butyrophenone; ○ = valerophenone; ▲ = hexanophenone.

approximately 37% with 60 m*M* SB-12 added. The selectivity for pair B-Bz, on the other hand, shows approximately a 46% increase in selectivity with the addition of 5 m*M* SB-12 and almost a 175% increase with the addition of 60 m*M* SB-12. These changes in selectivity could be the result of electrostatic interactions between the free electrons of the carbonyl group on benzophenone and the positively charged moiety of SB-12 on the surface of the micelle. If there are electrostatic interactions taking place between benzophenone and SB-12, it would result in decreased micelle penetration for benzophenone and increased mobile phase interactions, which in turn would decrease retention. This type of interaction could explain the decrease in retention seen for benzophenone and the selectivity changes.

### 3.6. *Efficiency*

Efficiencies were calculated using Eq. 3 for both the homologous series of alkylphenones and the neutral test analytes. Fig. 5 shows the efficiencies, averaged over five runs, for selected alkylphenones as a function of SB-12 concentration, at a constant concentration of 20 m*M* for SDS. As can be seen, the efficiencies for the alkylphenones show a substantial increase with each addition of [SB-12]. At a concentration of 60 m*M* SB-12, efficiencies were almost five times that which could be generated when only SDS is used. Although moderate increases in *N* are commonly observed for pure SDS systems as the SDS concentration is increased from 20 m*M* to higher values, the increases were not nearly as significant as with the mixed SDS–SB-12 system described here. Moreover, these moderate increases in *N* for the pure SDS systems come at the expense of much higher currents (Joule heating), in contrast to the much larger increases in *N* with no increases in current for the SDS–SB-12 system.

A similar trend is shown in Fig. 6 for other neutral test analytes. Although benzyl alcohol shows little increase in efficiency, this can be attributed to its very hydrophilic nature. Benzyl alcohol primarily experiences surface interactions with the mixed micelles and hence would not benefit very much from any type of efficiency enhancement generated with the SB-12–SDS system. The analytes that penetrate deeper into the mixed micelle stand to benefit the most. Clearly, both Figs. 5 and 6 illustrate the signifi-

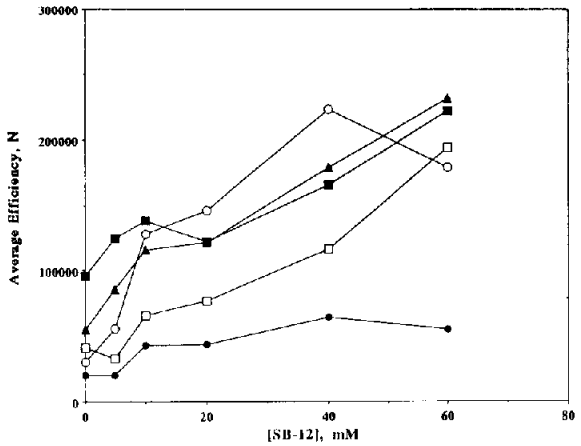


Fig. 6. Effect of micellar composition on efficiency for the neutral test analytes. Conditions as in Fig. 5. ● = Benzyl alcohol; ▲ = nitrobenzene; □ = anisole; ○ = benzophenone; ■ = decanophenone.

cant advantage gained by using the SB-12–SDS mixed micelles as a pseudostationary phase compared to pure SDS micelles.

The efficiency enhancement seen with the SB-12–SDS mixed micellar system can probably be attributed to: (i) better mass transfer kinetics between the analyte and micelle and (ii) less diffusional broadening. Although reasons for (i) are beyond the scope of the present work, (ii) can be briefly explained in terms of the apparent diffusion coefficient of an analyte in MEKC,

$$D_{app} = \left(\frac{1}{1+k'}\right)D_{analyte} + \left(\frac{k'}{1+k'}\right)D_{mc} \qquad (5)$$

where $D_{app}$ is the effective diffusion coefficient of the analyte, $D_{analyte}$ is the diffusion coefficient of the solute in free solution and $D_{mc}$ is the diffusion coefficient of the micelle. Since $D_{mc}$ is typically one to two orders of magnitude lower than $D_{analyte}$ for small to moderate-size molecules, a significant increase in $k'$ with minimal changes in migration time results in a decrease in $D_{app}$, and therefore less band broadening by longitudinal diffusion ($\sigma^2 = 2D_{app}t$).

Eq. 5 was derived using a phenomenological model that is equally valid for pure and mixed micellar systems. Given the much greater increases in efficiency with increasing surfactant concentration observed for a given analyte for the SDS–SB-12 micellar system compared to the pure SDS system, improvements in $N$ obviously cannot be attributed primarily to decreases in diffusional broadening via Eq. 5 unless $D_{mc,SDS-SB-12} \ll D_{mc,SDS}$, which seems unlikely.

### 3.7. Resolution

Ultimately a separation is judged by the extent of resolution that can be achieved. Previous studies in MEKC regarding the optimization of resolution and control of the hydrophobic character of the micellar system include the use of cyclodextrins [35], bile salts [36] and alkylglucoside–borate surfactants [37].

The bottom chromatogram shown in Fig. 7 compares the separation of the neutral test analytes using 20 m$M$ SDS to that achieved with 10 m$M$ SB-12–20 m$M$ SDS. It should be noted that the electrophoretic mobility of the micelle and electroosmotic flow were relatively unchanged with the addition of 5–20 m$M$ SB-12. This can be seen in Figs. 7 and 8 as the $t_{mc}$ and $t_0$ values do not change significantly ($< 15\%$). The most glaring contrast between the two separations is the baseline resolution that is obtained for the two structural isomers, $p$-nitrotoluene and $m$-nitrotoluene, with the SB-12–SDS system. The resolution of the two geometrical isomers were calculated using Eq. 2. The resolution for the two structural isomers was approximately 0.79 in 20 m$M$ SDS and 1.49 in 10 m$M$ SB-12–20 m$M$ SDS. This represents an almost 50% improvement in resolution with the addition of 10 m$M$ SB-12. Fig. 9 displays the separation obtained when 30 m$M$ SDS was used with no SB-12 added. Even at this higher concentration of SDS, baseline resolution of the two geometrical isomers could not be obtained. Furthermore, no significant improvement in $N$ from Fig. 7 (top) to Fig. 9 was observed, which corroborates our earlier discussion regarding increases in $N$ with increasing SDS concentration for pure SDS systems.

The chromatogram in Fig. 8 displays a separation of the alkylphenone homologous series.

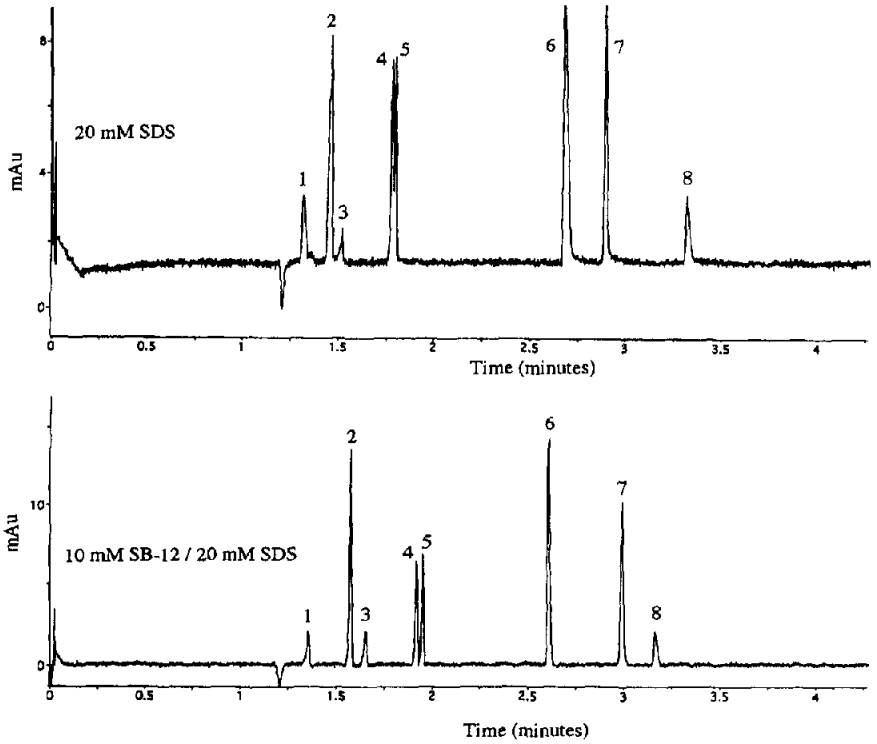


Fig. 7. Comparison of the separation of the neutral test analytes (Fig. 1) achieved with 20 m*M* SDS (top) and 10 m*M* SB-12–20 m*M* SDS (bottom). Baseline resolution of the two geometrical isomers, *p*-nitrotoluene and *m*-nitrotoluene, is obtained with the mixed SB-12–SDS system but not with SDS alone. Conditions as in Fig. 3. Peaks: 1 = benzyl alcohol; 2 = nitrobenzene; 3 = anisole; 4 = *p*-nitrotoluene; 5 = *m*-nitrotoluene; 6 = benzophenone; 7 = biphenyl; 8 = decanophenone.

The peaks are much sharper (and somewhat taller) with the mixed micellar system of 10 m*M* SB-12–20 m*M* SDS; given the only minor reductions in analyte migration time, the efficiencies are obviously higher, as is the signal-to-noise ratio (since the noise did not increase upon the addition of SB-12). Another more subtle but important advantage of the SB-12–SDS system is the slight improvement in resolution that can be seen for the most hydrophobic analytes, the $C_{14}$ and $C_{16}$ alkylphenones. The resolution of very hydrophobic solutes has been difficult in MEKC as these types of compounds tend to stay in the hydrophobic core of micelle and elute at or very near $t_{mc}$. The improvement in the resolution of the very hydrophobic analytes with the SB-12–SDS system represents another significant advantage of this system. The addition of organic modifiers to SB-12–SDS could result in better resolution for hydrophobic analytes without the high losses in efficiencies that accompany the use of organic modifier with SDS micelles.

### 3.8. Elution range effects

Monitoring the effects on the elution range ($t_{mc}/t_0$) is important in terms of peak capacity and optimization of resolution. A reduction in the magnitude of the elution range is detrimental in terms of decreased peak capacity. However, if the elution range does not change significantly this could facilitate the optimization of resolution. Table 5 lists the $t_{mc}/t_0$ values obtained at different [SB-12]–20 m*M* SDS. The elution range did not change significantly with the addition of 5–20 m*M* SB-12, but did decrease significantly upon the addition of 40–60 m*M* SB-12. The best separations were achieved with con-

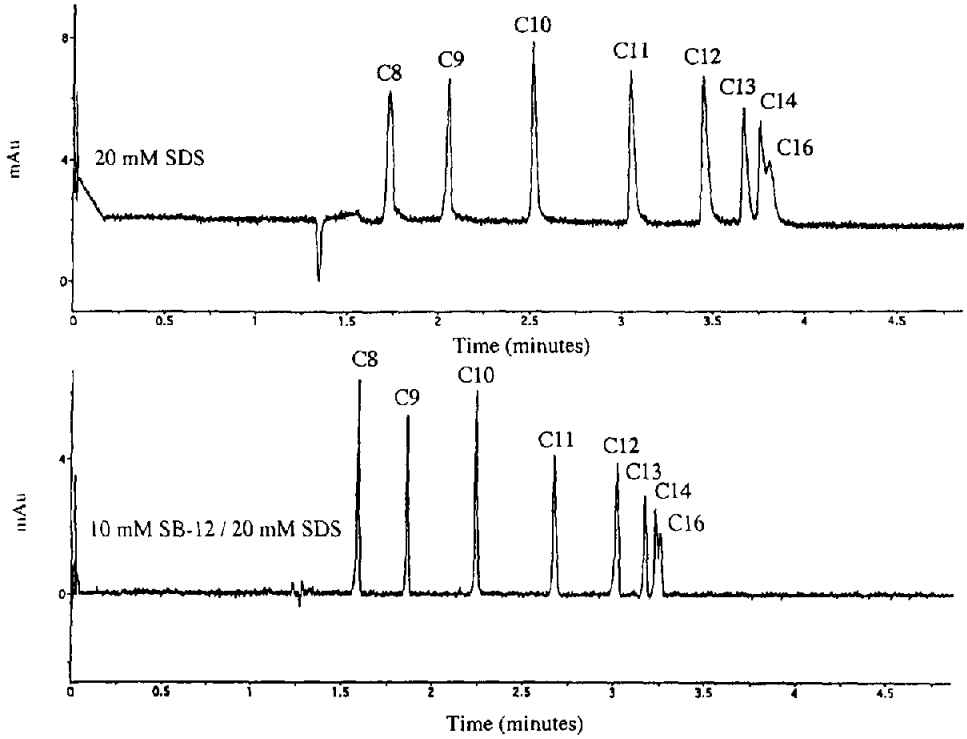


Fig. 8. Comparison of alkylphenone separation with 20 m*M* SDS (top) and 10 m*M* SB-12–20 m*M* SDS (bottom). Note: the peaks are much sharper for the mixed micellar system and consequently there is an improvement in resolution for the most hydrophobic analytes, the $C_{14}$ and $C_{16}$ homologues. Conditions as in Fig. 3.

centrations of 10–20 m*M* SB-12. This is not surprising because the efficiency enhancements and selectivity improvements afforded through the use of SB-12 were gained without a significant loss in peak capacity.

One interesting characteristic of the SB-12–SDS system is that the electroosmotic flow did not change with the addition of 5–20 m*M* SB-12 but did change significantly when higher concentrations of SB-12 were used. Fig. 10 shows the absolute electrophoretic mobility ($\mu_{abs}$) of the SB-12–SDS micelle at various ratios of the

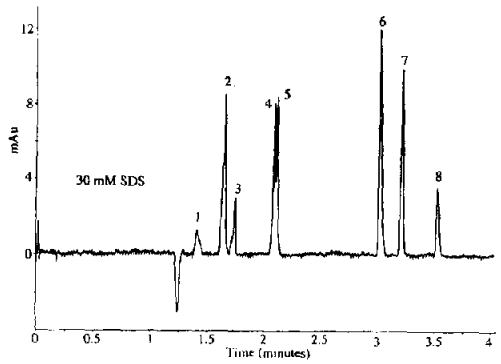


Fig. 9. Separation of the neutral test analytes shown in Fig. 1 with 30 m*M* SDS. Applied voltage: 20 kV; operating currents: $< 45\ \mu A$; capillary length: 30 cm (injection to detection); 3 s hydrostatic injection; temperature: ambient; 10 m*M* phosphate buffer pH 7.0. Peaks as in Fig. 7.

Table 5
Elution range for the [SB-12]–20 m*M* SDS system

| [SB-12] (m*M*) | $t_{mc}/t_0$ |
|---|---|
| 0 | 2.86 |
| 5 | 2.69 |
| 10 | 2.65 |
| 20 | 2.64 |
| 40 | 2.45 |
| 60 | 2.06 |

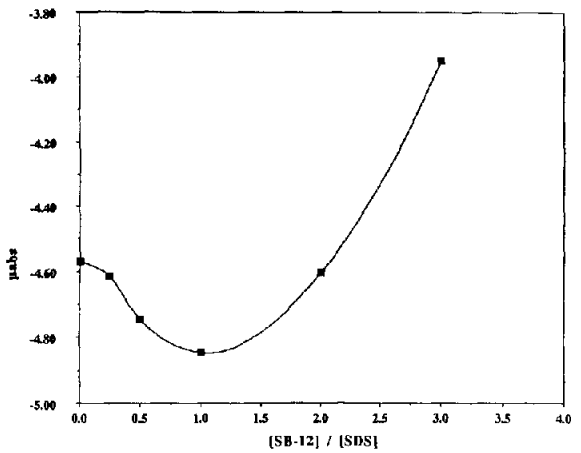


Fig. 10. Absolute electrophoretic mobility of the mixed micelle as a function of [SB-12]/[SDS] ratio. $\mu_{abs}$: $\times 10^{-4}$ $cm^2$ $V^{-1}$ $s^{-1}$.

two surfactants. The magnitude of $\mu_{abs}$ decreases sharply with the addition of 40–60 m*M* SB-12. This decrease in the electrophoretic mobility of the micelle, despite a minor decrease in electroosmotic flow, explains why the elution range decreases significantly at these concentrations.

## 4. Conclusions

The results of this investigation further support the need to investigate the use of mixed micellar pseudostationary phases in MEKC. The degrading effects of using additives such as organic modifiers to improve resolution can probably be avoided in many cases by using mixed micelles. Micellar structure seems to play a significant role in the overall column efficiency that can be generated. Judicious choice of a micellar system for use as a pseudostationary phase is extremely important and should be made with respect to type of analytes that are to be separated. The SB-12–SDS mixed micellar system should be very good for the analysis of hydrophilic to moderately hydrophobic compounds. Very hydrophobic analytes will also benefit albeit to a lesser degree.

Several advantages of the SB-12–SDS micellar system were observed. First, 2–5-fold increase in column efficiency was obtained with the range of SB-12 concentrations employed. Second, unique selectivity variations were seen for moderately hydrophobic neutral analytes. Third, baseline resolution of the two structural isomers studied can be obtained with a 10–20 m*M* SB-12–20 m*M* SDS which could not be obtained when the pseudostationary phase consisted of only SDS micelles. Fourth, the stability of the elution range aided in resolution optimization. Fifth, repeatability of the separations were very good as no significant fluctuations in the migration times of the analytes were detected (R.S.D. < 1.5%). This represents a significant advantage over the anionic–non-ionic SDS–Brij 35 mixed micellar system previously studied. Finally, the use of SB-12 greatly reduced the possibility of Joule heating and hence the risk of capillary overheating.

An optimum concentration range of 10–20 m*M* SB-12 in combination with 20 m*M* SDS is recommended in order to achieve the best possible separations with this novel mixed micellar system.

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