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On-line sample preconcentration in micellar electrokinetic chromatography by sweeping with anionic–zwitterionic mixed micelles

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Abstract

On-line preconcentration by sweeping in micellar electrokinetic chromatography using mixed micelles of sodium dodecyl sulfate (SDS)–SB-12 is presented. Because of their large micelle radius, they permit increased partitioning of hydrophobic analytes into the core. In addition, they also possess lower negative surface charge relative to pure SDS micelles so anionic analytes can be retained better due to decreased electrostatic repulsion. As the efficiency of sweeping is predicated on the magnitude of retention factors, these advantages translated to better focusing. As much as a 370-fold improvement in detector response, in terms of peak height, was obtained for some neutral steroids, while about a 360-fold improvement was obtained for some phenol derivatives, which were previously not amenable to sweeping by pure SDS micelles.

Keywords: Sample handling; Sweeping; Mixed micelles; Steroids; Carboxylic acids; Phenols

1. Introduction

In capillary electrophoresis (CE), injected sample volumes are typically limited to 1% of the total capillary volume in order to maintain efficiency [1]. As the internal diameter of capillaries employed for routine applications is generally less than 100 μm, and only a short path length is available for on-line optical detection, CE suffers from poor concentration sensitivity. In response to this problem, on-line preconcentration techniques, which enable loading of large amounts of sample and subsequent narrowing of sample zones prior to separation, have been developed. These include field-enhanced sample stacking (FESS) [2–9], large volume sample stacking (LVSS) [10–15], acetonitrile stacking [16–18], pH-mediated stacking [19–25], and sweeping [26–34], to name a few. Most of these techniques are grounded on the use of discontinuous electrolyte systems. Sample matrices are so designed that they differ from background solutions (BGS) in terms of conductivity, ionic strength, pH, or the absence of some additives. As such, analyte molecules possess different velocities in these regions, and it is this disparity in velocities which is exploited to cause them to focus into narrow bands [24].

Sweeping, one of the newer strategies, is based on the accumulation of analyte molecules by an additive in the BGS which they have a considerable affinity for [26]. Although the range of buffer additives has been extended to include charged cyclodextrins [29]

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and complexing agents [30], by and large, the most common sweeping agents used are micelles. The general condition is that the sample matrix be devoid of the micelles. Upon application of voltage, analyte–micelle interaction is initiated, and the magnitude of this interaction largely determines the extent of focusing. The length of the sample injection plug, l_{inj}, is predicted to be narrowed depending on the retention factor, k, to give the length of the swept zone, l_{sweep}, as shown in Eq. (1) [26]:

$$l_{sweep} = l_{inj} \cdot \left(\frac{1}{1+k} \right) \quad (1)$$

This implies that the more strongly retained an analyte is, the shorter its band width will be, and the more efficiently it can be focused.

In this report, we evaluate the effectiveness of an anionic–zwitterionic mixed micelle system for sweeping. To the best of our knowledge, such a system has not been exploited previously in the context of preconcentration, although the concept of 'mixed micelle' itself is not new. In the parlance of micellar electrokinetic chromatography (MEKC), the phrase 'mixed micelle' means a micelle composed of different surfactants, each of which is capable of forming micelles [35].

In MEKC, there have been numerous reports on the use of mixed micelles for varying selectivity. An optimized system composed of sodium dodecyl sulfate (SDS)–Brij 35 was reported for the separation of herbicides [36], phenylthiohydantoin (PTH)-amino acids [37], benzene derivatives [38], and pyridine and chloropyridines [39]. The advantages of an SDS–sodium cholate (SC) mixed system over a pure SDS system for the separation of corticosteroids [40], and benzophenones [41] have been demonstrated. Chiral surfactants such as bile salts, dodecoxycarbonylvaline (DDCV) and sodium *N*-dodecanoyl-L-valinate (SDVal) were added to nonchiral surfactants to improve enantiomeric resolution [42–45]. A mixture of SDS with the oppositely charged dodecyltrimethylammonium bromide (DTAB) has been shown to provide larger methylene selectivity for a series of alkyl phenyl ketones relative to pure SDS [46]. More recently, a method for quantitative analysis of bacitracin using a combination of the zwitterionic 3-(*N*,*N*-dimethylhexadecylammonium)-propanesulfonate (PAPS) with Brij 35 was developed [47].

When SDS was combined with the zwitterionic *N*-dodecyl-*N*,*N*-dimethylammonium-3-propane-1-sulfonic acid (SB-12) [48] and Brij 35 [38,49,50], substantial increases in the retention factors of an array of neutral analytes were noted. It is this advantage which we explored in this work, and as such is expected to be favorable for sweeping.

Combining SDS with a net zero charge surfactant will result in a mixed micelle with a negative charge. Hence, in the presence of high electroosmotic flow (EOF), the sweeping model proposed by Isoo et al. [33] was adopted. To wit, the capillary is initially conditioned with micellar BGS; then, a long plug of sample, prepared in nonmicellar buffer of approximately equal conductivity to the BGS, is introduced hydrodynamically. When voltage is applied, analyte molecules at the front end of the sample plug are trapped by the more slowly moving micelles; hence, their migration velocities are reduced. Molecules at the rear end travel more quickly until they, too, are incorporated into the micelles, and the whole sample region is compressed. After sweeping is completed, the focused zones are subsequently separated and brought to the detector according to the MEKC principle.

To avoid the capillary coating effect considered to be a disadvantage with an SDS–Brij 35 system, an SDS–SB-12 combination was selected. Neutral steroids, carboxylic acids, and phenol derivatives were used as model analytes.

2. Experimental

All CE experiments were carried out with a Beckman P/ACE 2000 System (Fullerton, CA, USA) or a Hewlett-Packard ^{3D}CE System (Waldbronn, Germany) equipped with fused-silica capillaries from Polymicro Technologies (Phoenix, AZ, USA) with effective length of 50 cm or 30 cm and I.D. 50 μm, thermostated at 25 °C. Pressure injections were carried out at 3.5 kPa (Beckman instrument) or at 5 kPa (HP instrument).

As a conditioning regimen, newly installed capillaries were flushed successively with 1 *M* NaOH (30 min), methanol (30 min), and water (30 min). To

ensure reproducibility between consecutive analyses, the capillaries were flushed with 1 *M* NaOH (1 min), methanol (1 min), water (5 min), and BGS (5 min).

In order to approximate the length of the sample injection plug, the velocity of the liquid was estimated by injecting a neutral marker and measuring the time required to bring it to the detector using the same pressure used for typical injections. Conductivities were measured with a Horiba ES-12 conductivity meter (Kyoto, Japan). The pH of solutions were measured and adjusted with the aid of a Beckman ϕ 34 pH meter. Water was purified with a Milli-Q system from Millipore (Bedford, MA, USA).

Reagents of the highest grade available were used. The phenol derivatives, steroids, and SDS were from Nacalai Tesque (Kyoto, Japan); sudan III was from Wako (Osaka, Japan); disodium hydrogen phosphate was from Kanto (Tokyo, Japan); sodium tetraborate decahydrate was from Fluka (Buchs, Switzerland); SB-12, and tetradecyltrimethylammonium bromide (TTAB) were from TCI (Tokyo, Japan). Stock solutions of phosphate, borate, and micelles were prepared in purified water. The micellar buffer solutions were made by mixing the stock solutions of phosphate or borate with SDS and SB-12 in suitable volumes to come up with the desired concentration, and the pH of the resulting solutions were adjusted accordingly thereafter. Stock solutions of the phenols were prepared in purified water or 50% methanol. The steroids were prepared in methanol. Sample matrices were prepared by diluting the appropriate amount of the stock solutions with nonmicellar buffer solutions, which were previously adjusted to the desired conductivity by titration with higher or lower concentration buffer solutions. All solutions were passed through 0.45-μm filters from Nacalai Tesque (Kyoto, Japan) prior to use.

3. Results and discussion

3.1. Some characteristics of SDS–SB-12 mixed micelles

Schematic diagrams of SDS, SB-12, and SDS–SB-12 mixed micelles are shown in Fig. 1. In an SDS–SB-12 mixed micelle, the anionic–cationic head groups of SB-12 are interspersed between the anionic head groups of SDS, leading to weakened electrostatic repulsion between the latter. Since less electrical work is required to form the micelle, the critical micellar concentration (CMC) is lower than that predicted for an ideal mixture [51,52]. While its hydrophobicity is not expected to be significantly different from the component surfactants because of

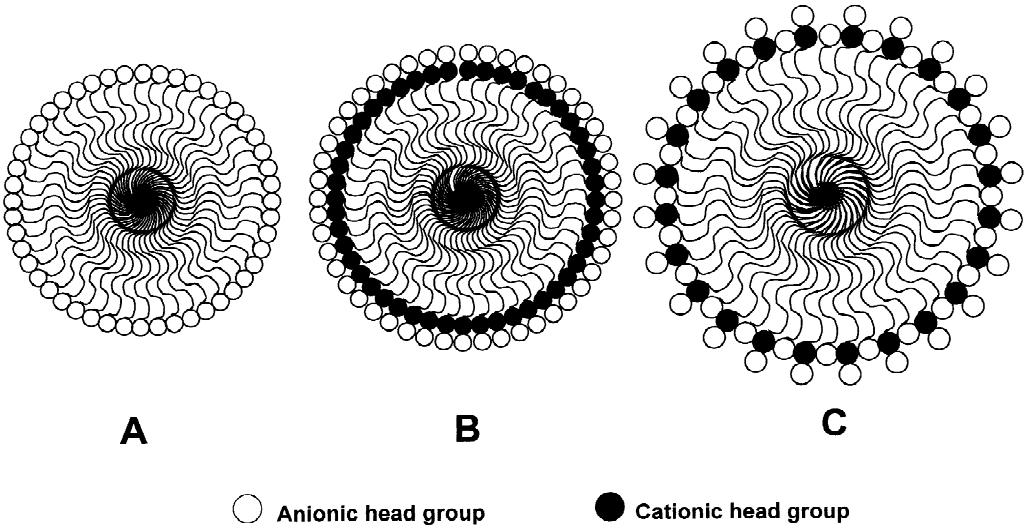

Fig. 1. Schematic diagrams of anionic SDS (A), zwitterionic SB-12 (B), and SDS–SB-12 mixed micelles (C).

identical alkyl chain lengths, the size of the micellar radius is projected to be increased [48].

A mixed micelle of an ionic–nonionic surfactant is known to have a larger micelle radius compared to an ionic micelle [49,53]. The same may be expected of a combination of an ionic surfactant with another net zero charge surfactant such as the zwitterionic SB-12. In fact, micellar self-diffusion data from mixed micelles of lithium dodecyl sulfate (LDS)–SB-12 indicate a concomitant increase in micelle radius with increasing proportion of SB-12 in the mixture [48,54].

Another consequence of incorporation of SB-12 head groups between those of SDS is surface charge ''dilution'', which in turn profoundly affects the electrophoretic mobility of the mixed micelle. In this work, the effective electrophoretic mobility of the micelle, $\mu_{mc,\ eff}$ was calculated by Eq. (2):

$$\mu_{mc,\ eff} = \mu_{EOF} - \mu_{mc,\ app} \quad (2)$$

where μ_{EOF} is the mobility of the EOF, and $\mu_{mc,\ app}$ is the apparent mobility of the micelle, with a negative sign since its electrophoretic migration is directed against the former. Methanol and sudan III were used as markers of the EOF and the micelle, respectively.

Fig. 2 shows the trend in effective micelle mobility with increasing surfactant concentration, in addition to a base of 20 m*M* SDS. Within the range studied, no significant change was observed for the pure SDS system. Increasing the surfactant concentration will increase the system temperature, which in turn will accelerate migration velocities. However, this effect is dampened by an attendant rise in viscosity. In contrast, the effective mobility of the mixed micelle decreased with increasing proportion of SB-12. (Note that no data were obtained at combinations of 20 m*M* SDS with less than 30 m*M* SB-12 because the resulting solutions were of such viscosities that loading them into the capillaries was difficult and results were not reproducible.) As the fraction of SB-12 mounted, SDS head groups were thrown progressively apart, resulting in surface charge dilution. Hence the mixed micelle may be said to bear a lower negative surface charge, and

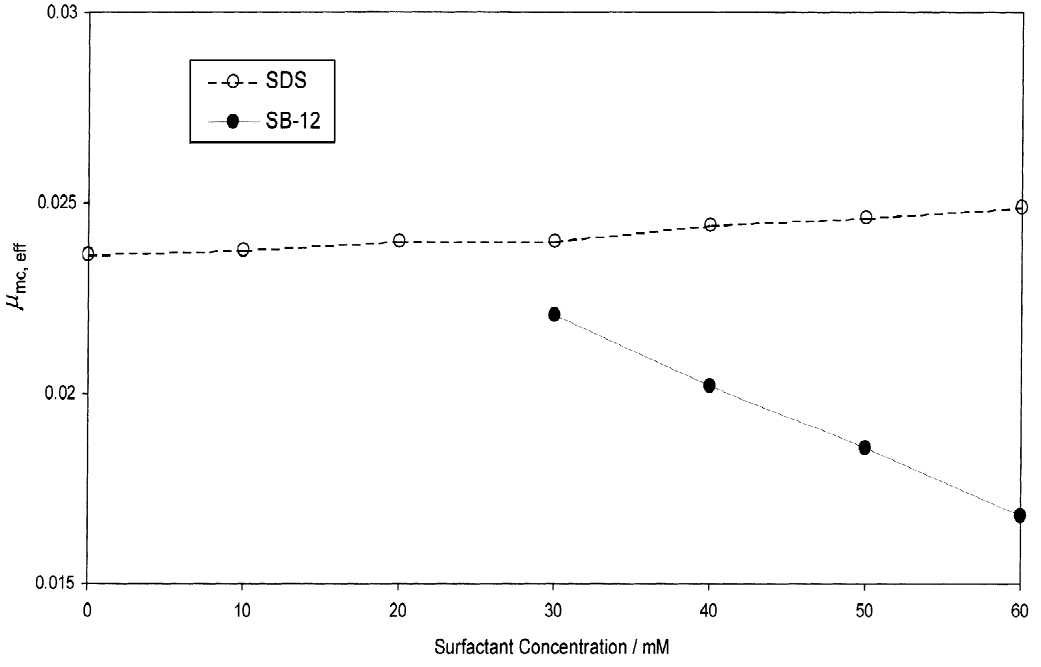

Fig. 2. Plot of surfactant concentration in addition to 20 m*M* SDS in 15 m*M* borate, pH 11.0 versus effective mobility of the micelle. Conditions: fused silica capillary, effective length of 50 cm, total length of 58.5 cm; applied voltage, +20 kV; detection, 200 nm. Other conditions are the same as given in the Experimental section.

exhibited less resistance to the EOF. While this was thought to account primarily for the decrease in effective micelle mobility, the increase in micelle radius should not be discounted as well.

Anionic analytes are not retained strongly by anionic micelles because of electrostatic repulsion. Hence, charge moderation is expected to favor increased retention. In this study, retention factor, k, for the anion, a′, was calculated by Eq. (3) [55,56]:

$$k = \frac{\mu_{\mathrm{MEKC(a')}} - \mu_{\mathrm{CZE(a')}}}{\mu_{\mathrm{mc}} - \mu_{\mathrm{MEKC(a')}}} \quad (3)$$

where $\mu_{\mathrm{MEKC(a')}}$ and $\mu_{\mathrm{CZE(a')}}$ are the mobilities of the analyte in MEKC and CZE modes, respectively, and μ_{mc} is the mobility of the micelle. k is a function of both the distribution coefficient of the analyte between the aqueous phase and the micellar pseudophase, K, and the phase ratio $V_{\mathrm{mc}}/V_{\mathrm{aq}}$, where V_{mc} and V_{aq} are the volumes of the micelle and the remaining aqueous phase as shown in Eq. (4) [53]:

$$k = K \cdot \frac{V_{\mathrm{mc}}}{V_{\mathrm{aq}}} \quad (4)$$

Alternatively, Eq. (4) may be written as:

$$k = K\bar{v}(C_{\mathrm{sf}} - \mathrm{CMC}) \quad (5)$$

where $\bar{v}$ is the partial specific volume of the micelle, and C_{sf} is the concentration of the surfactant. An obvious implication of this equation is that k increases linearly with an increase in surfactant concentration [53].

Fig. 3 shows the trend in retention factor of three model alkylphenols upon progressive addition of SDS and SB-12 to a base of 20 m*M* SDS in the BGS. In the pure SDS system, the increase may be attributed solely to an increasing phase ratio. In the binary system, however, both the phase ratio and the distribution coefficient are affected, which could account for the higher retention factors obtained [48]. In general, an analyte can interact with a micelle by penetration into the alkyl core, in the case of hydrophobic species; or by adsorption onto the polar surface, in the case of more hydrophilic ones. Under the conditions employed, the dissociated moieties of the phenols predominated. Since the mixed micelle bore more moderate negative charge,

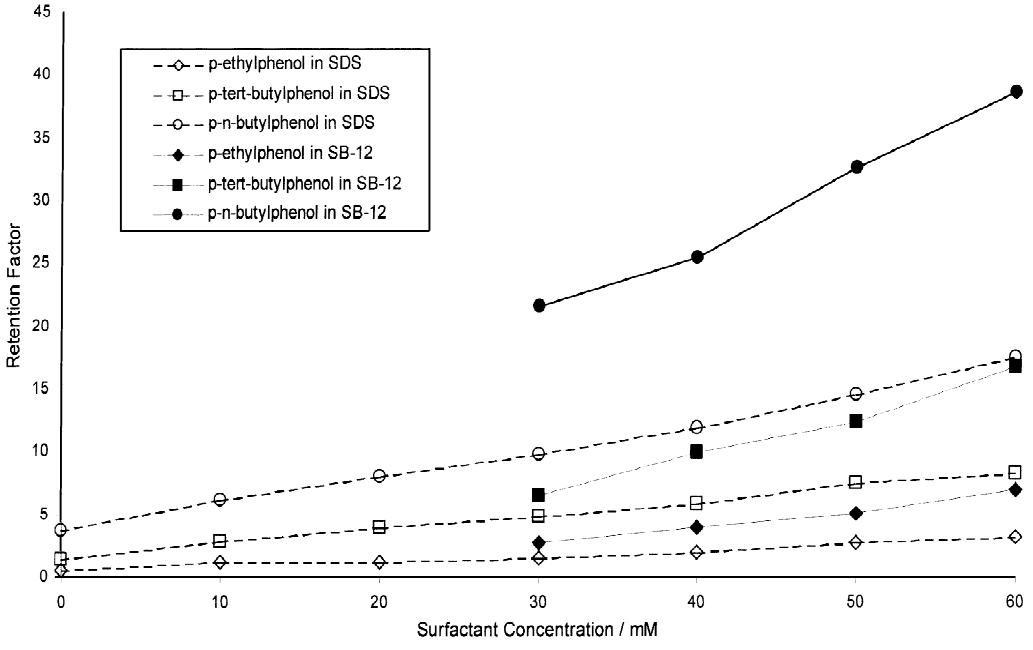

Fig. 3. Plot of surfactant concentration in addition to 20 m*M* SDS in 15 m*M* borate, pH 11.0 versus retention factor. Other conditions are the same as given in Fig. 2.

the anionic phenols were reasonably well retained. Being hydrophobic, the interaction involving solubilization in the core was amplified as well. In a previous study with SDS–SB-12 [48] system, the observed increases in the retention factors of model neutral analytes were ascribed to the increase in micelle radius, permitting more hydrophobic analytes to be in the ''dissolved'' state in the core.

3.2. Sweeping of neutral analytes

With a larger micelle radius, more hydrophobic analytes may be partitioned into the core, resulting in increased retention. This, in turn, will be advantageous for sweeping.

Fig. 4A is a typical electropherogram resulting from the injection of a mixture of three neutral steroids under conventional MEKC conditions, while Fig. 4B is from a 200-fold dilution of the same, injected under sweeping conditions. Detector responses were improved by a factor of 320, 330 and 370 for hydrocortisone, testosterone and progesterone, respectively. These values are better than those reported for similar test analytes using pure anionic [27] or pure cationic [32] systems in the presence of high electroosmotic flow.

3.3. Sweeping of anionic analytes

Fig. 5A,C is from conventional MEKC injections of mixtures of benzoic and salicylic acids (Fig. 5A), and 2,4,6-trichlorophenol and *p-tert.*-butylphenol (Fig. 5C). Corresponding electropherograms (Fig. 5B,D, respectively) obtained under sweeping conditions show that focusing of these analytes with SDS was not very successful. In terms of sensitivity enhancement factor by peak height, SEF_{height}, the carboxylic acids and 2,4,6-trichlorophenol were hardly focused at all, while *p-tert.*-butylphenol focused to an extent to 30-fold at best.

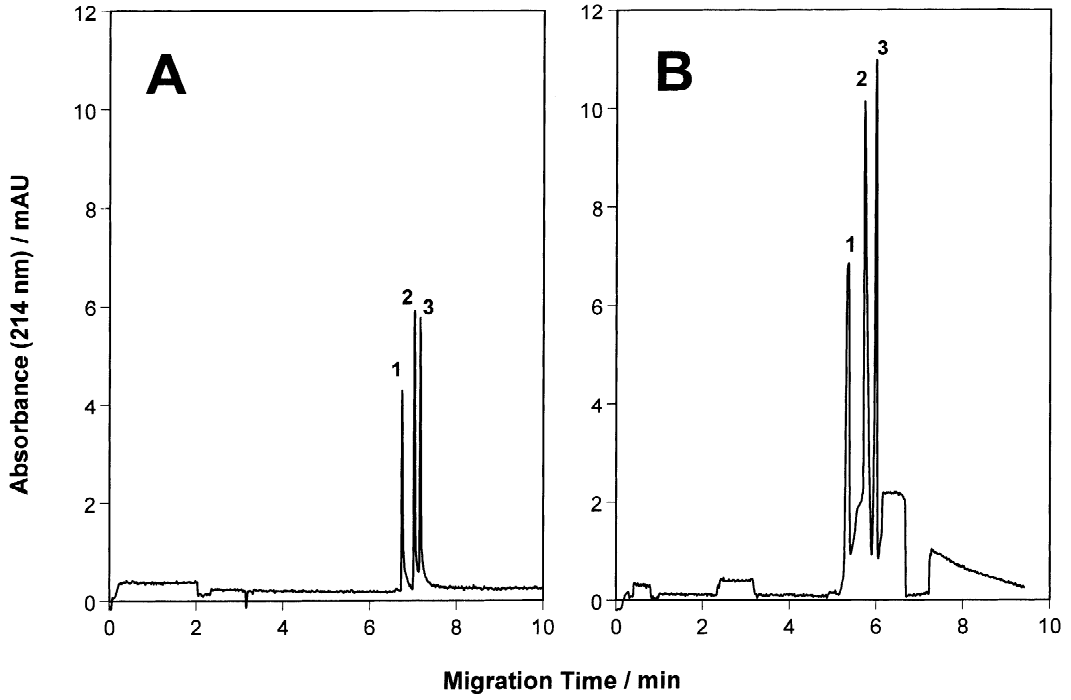

Fig. 4. MEKC separation of neutral steroids. Conditions: BGS, 10 m*M* phosphate–20 m*M* SDS–60 m*M* SB-12, pH 7.0; applied voltage, +25 kV; injection plug length 0.6 mm (A), and 12.3 cm (B); sample concentration, 300 ppm each (A), and 1.5 ppm each (B); samples, hydrocortisone (peak 1), testosterone (peak 2), and progesterone (peak 3). Other conditions are the same as given in the Experimental section.

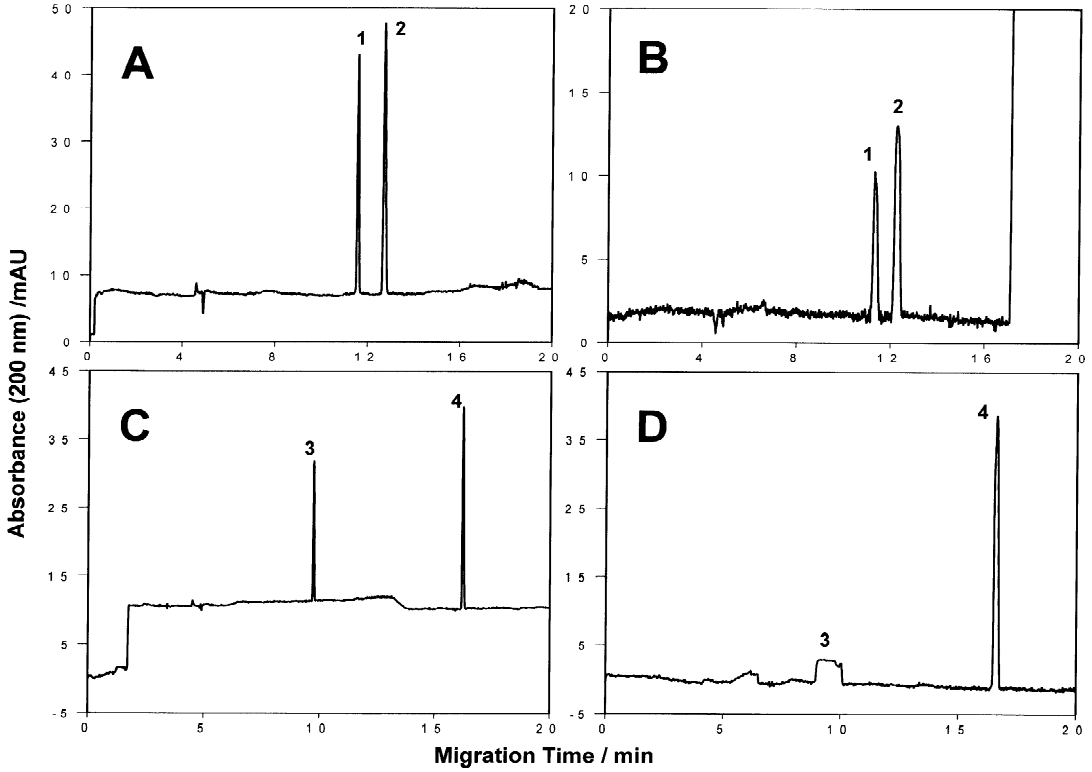

Fig. 5. MEKC separation of anionic test analytes using SDS. Conditions: BGS, 15 m*M* borate–80 m*M* SDS, pH 11.0; applied voltage, +20 kV; injection plug length, 0.7 mm (A and C), 7.0 mm (B), and 4.1 cm (D); sample concentration, 180 ppm each (A and C), 18 ppm each (B), and 9 ppm each (D); samples, benzoic acid (peak 1), salicylic acid (peak 2), 2,4,6-trichlorophenol (peak 3), and *p-tert.*-butylphenol (peak 4). Other conditions are the same as given in the Experimental section.

When an SDS–SB-12 mixed micelle system was substituted for pure SDS (Fig. 6), significant improvement in sweeping efficiency was obtained. As compared to conventional MEKC injections (Fig. 6A,C), peak heights were improved by a factor of 20, 60, 120 and 360 for benzoic acid, salicylic acid, *p-tert.*-butylphenol and 2,4,6-trichlorophenol, respectively, when dilutions of these test analytes were injected under sweeping conditions. For the phenols, linearity of peak height response against concentration and reproducibility of other peak descriptors were found satisfactory, and limits of detection were in the low ppb levels (Table 1).

3.4. Comparison with zwitterionic and cationic micelle systems

Despite the considerable hydrophobicity of the test phenols, they still did not focus well when swept using SDS. As electrostatic interaction is known to be much stronger than hydrophobic interaction, it may be said that the effect of the latter was not enough to compensate for the electrostatic repulsion between the similarly charged analyte and micelle. To obtain good results, a logical approach, therefore, is to supplant the anionic micelle with a net zero charge system, such as a zwitterionic type, to altogether avoid the deleterious effect of charge repulsion; or better yet, a cationic type, to even promote electrostatic attraction between the analyte and the micelle.

When the same test analytes were swept using SB-12 (Fig. 7), the results were inferior to those obtained using SDS–SB-12 mixed micelle, i.e. the detector response was improved by a factor of only 50 at best. On the other hand, when the cationic

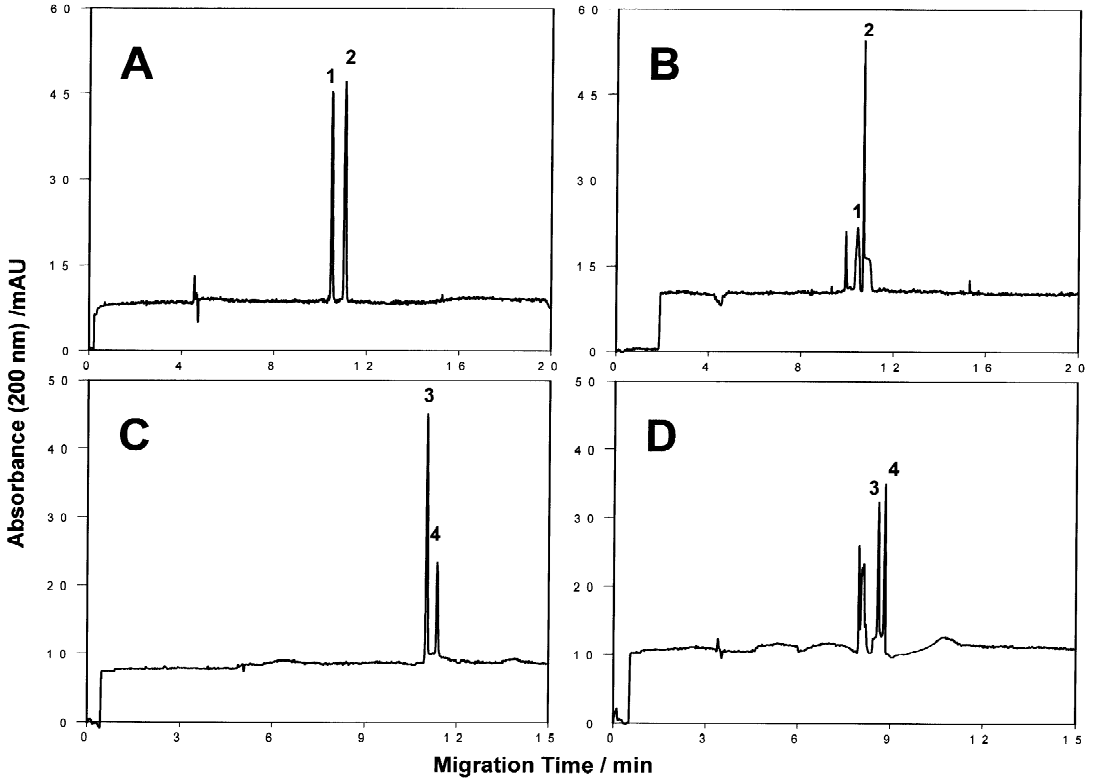

Fig. 6. MEKC separation of anionic test analytes using SDS–SB-12. Conditions: BGS, 15 m*M* borate–20 m*M* SDS–60 m*M* SB-12, pH 11.0; applied voltage, +20 kV; injection plug length, 0.7 mm (A and C), 2.8 cm (B), and 12.4 cm (D); sample concentration, 180 ppm each (A and C), 3.6 ppm each (B), and 900 ppb each (D); samples, benzoic acid (peak 1), salicylic acid (peak 2), *p-tert*.-butylphenol (peak 3), and 2,4,6-trichlorophenol (peak 4). Other conditions are the same as given in the Experimental section.

Table 1
Calibration line, limit of detection (LOD), % relative standard deviation, and sensitivity enhancement factor in terms of peak height (SEF_{height})

	Analyte	
	p-tert.-Butylphenol	2,4,6-Trichlorophenol
Calibration line		
Equation of the line[a]	$y=11.02x+2.17$	$y=13.65x+1.43$
Correlation coefficient, r^2	0.9991	0.9940
LOD (S/N=3)		
ppb	24	19
$\times 10^{-8}$ *M*	15.8	9.7
RSD (%, n=3)		
Migration time	0.5	0.5
Corrected peak area[b]	4.7	6.1
Peak height	2.1	7.3
SEF_{height}[c]	122	360

[a] Peak height (mAu) = slope × concentration (ppm) + *y*-intercept.

[b] Corrected peak area = peak area / migration time.

[c] $\mathrm{SEF_{height}} = \dfrac{\text{peak height with sweeping injection}}{\text{peak height with typical injection}} \times \text{dilution factor.}$

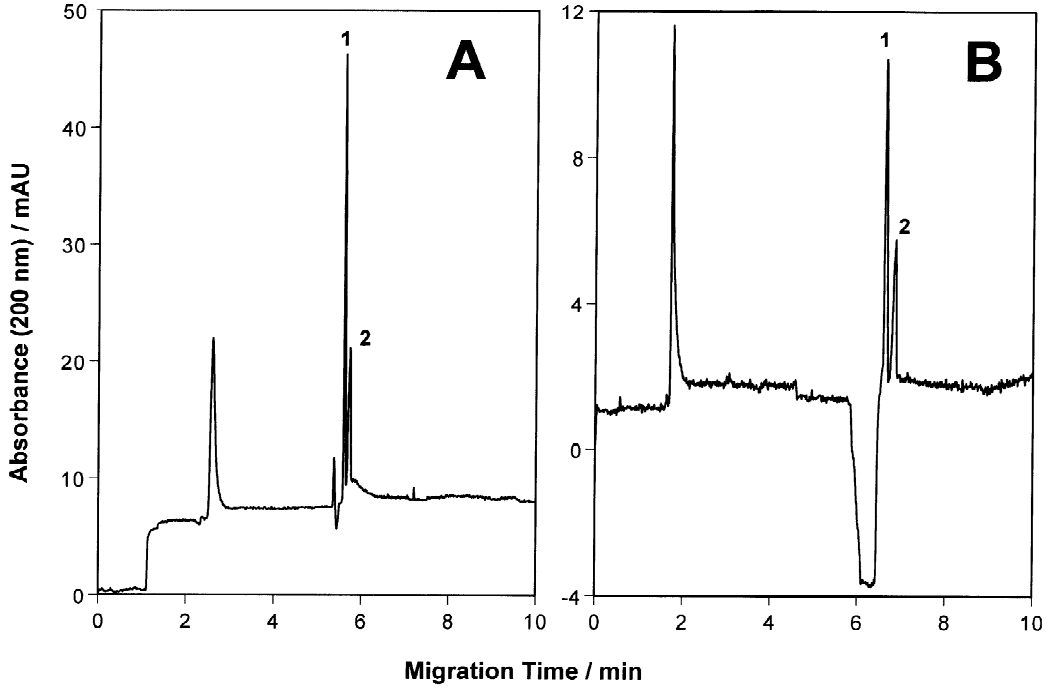

Fig. 7. MEKC separation of test phenols using SB-12. Conditions: BGS, 15 m*M* borate–80 m*M* SB-12, pH 11.0; applied voltage, +20 kV; injection plug length, 0.8 mm (A), and 4.8 cm (B); sample concentration, 180 ppm each (A) and 1.8 ppm each (B); samples, *p-tert*.-butylphenol (peak 1), and 2,4,6-trichlorophenol (peak 2). Other conditions are the same as given in the Experimental section.

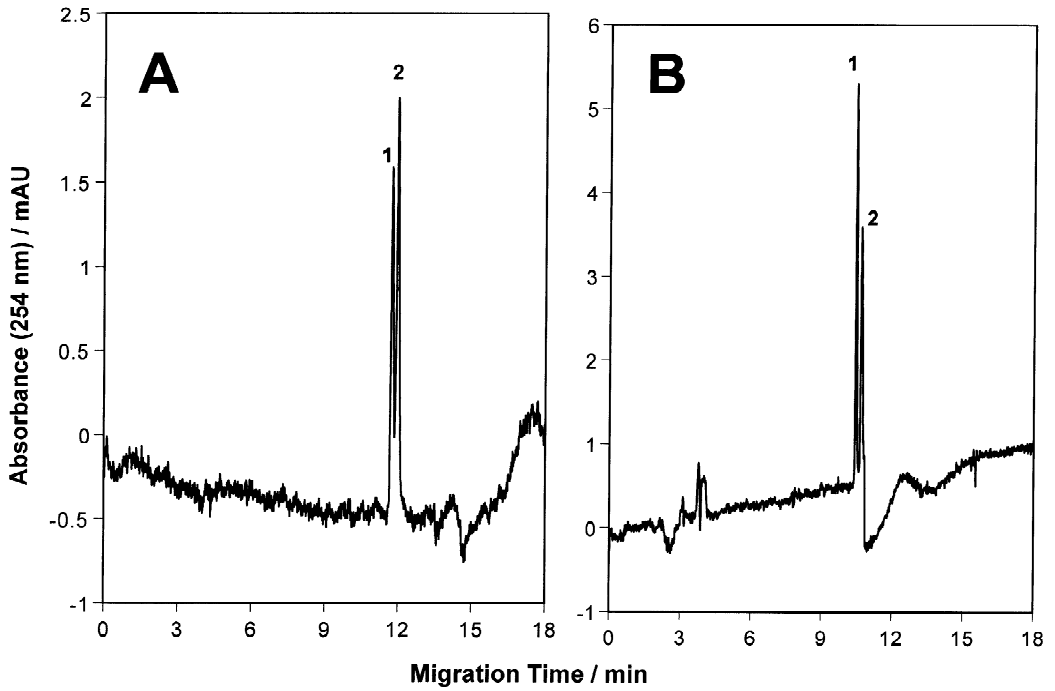

Fig. 8. MEKC separation of test phenols using TTAB. Conditions: BGS, 15 m*M* borate–80 m*M* TTAB, pH 11.0; applied voltage, −20 kV; injection plug length, 0.7 mm (A), and 4.1 cm (B); sample concentration, 150 ppm each (A), and 3.75 ppm each (B); samples, *p-tert*.-butylphenol (peak 1), and 2,4,6-trichlorophenol (peak 2). Other conditions are the same as given in the Experimental section.

TTAB was employed (Fig. 8), the enhancement in signal by a factor of 260 for *p-tert*.-butylphenol was better than that obtained using mixed micelle. 2,4,6-Trichlorophenol, however, was improved by a factor of only 120. These results suggest that the advantage of using an SDS–SB-12 mixed micelle system does not rest solely on its having a more moderate surface charge. The effect of large micelle radius, which enabled increased partitioning into the core, should not be trivialized as well.

4. Conclusion

We have demonstrated the usefulness of anionic–zwitterionic mixed micelles for on-line preconcentration by sweeping. These mixed micelles have the twofold advantage of having a large micelle radius and a moderate surface charge, which could work well for anionic, hydrophobic analytes. The use of the same has also extended the range of micellar additives in the BGS to less conventional systems. For sweeping, additives should be so chosen that optimum interaction with the analyte can be achieved, and focusing efficiency can be maximized. This will help bolster the concentration sensitivity of CE, and ultimately, improve its overall utility for more concrete applications.

Acknowledgements

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