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# Hydrogen-bonding interaction-assisted micellar electrokinetic chromatography using mixed surfactant systems

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**Abstract**

Micellar electrokinetic chromatography was examined using mixed surfactant systems consisting of Brij 35 or Tween 20, non-ionic surfactants with a polyether structure, together with sodium dodecyl sulfate (SDS). Addition of the non-ionic surfactant to a SDS micellar system provides a selective increase in the relative capacity factors of some substituted benzenes having hydrogen-donating substituents such as a hydroxyl, amino and amide groups. This effect can be ascribed to hydrogen bond formation between these solutes and the polyether segments of the non-ionic surfactant. The hydrogen-bonding interaction appears to work additively against the hydrophobic interaction. The separation selectivity can be well controlled by adjusting the mixing ratio of the two surfactants. The thermodynamic aspects of the mixed micellar system are discussed in detail.

*Keywords:* Buffer composition; Surfactants; Benzenes

## 1. Introduction

Micellar electrokinetic chromatography (MEKC) using ionic surfactant micelles as pseudo-stationary phases and carriers has been developed as one of the separation modes in capillary electrophoresis and is applicable to the separation of neutral and charged analytes [1,2]. The separation mechanism in MEKC is fundamentally based on the distribution of analytes between the pseudo- and aqueous phases. A variety of ionic surfactants have been examined to realize novel selectivity in MEKC. Sodium dodecyl sulfate (SDS) is most frequently used in MEKC, where the distribution is predominantly governed by the hydrophobic interaction between analytes and the hydrocarbon chain of SDS. Several "functional" moieties in other surfactant molecules can offer additional or cooperative interaction fields. Chiral surfactants such as sodium N-dodecanol-L-valinate [3–6], N-dodecanol-L-glutamate [7] and various bile salts [8–11] have been utilized for chiral separation. Anionic surfactants having polar moieties besides the charged head group exhibit unique separation selectivity, which is clearly different from that of SDS [12].

Such additional and/or specific interactions can be directly incorporated into the MEKC mode by utilization of mixed surfactant systems.

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For example, the anionic chiral surfactants mentioned above are coupled together with "achiral" SDS to improve separation efficiency and also peak shapes [6,8]. Mixed micelles composed of SDS and non-ionic chiral surfactants such as digitonin have been reported to provide "charged and chiral-selective" pseudo-stationary phases for chiral separation [5,7]. Incorporation of sodium 1-octane sulfate (SOS) into the SDS micelle produces a novel mixed micelle for the separation of catechols [13], in which SOS is different from SDS simply in the hydrocarbon chain length. A zwitterionic surfactant, N-dodecyl-N,N-dimethylammonium-3-propane-1-sulfonic acid, was used with SDS to improve column efficiency and separation selectivity [14]. A mixed micelle composed of SDS and Brij 35, a popular non-ionic surfactant, has been reported to improve separation selectivity [15–18]. This mixed surfactant system is also useful for increasing the elution range in MEKC [19].

In previous papers [20,21], a hydrogen-bonding mode capillary zone electrophoresis (CZE) using polyethylene glycol (PEG) as a matrix was reported. The polyether oxygen atoms of PEG appear to serve as hydrogen acceptors to form hydrogen-bonding complexes with analytes having hydrogen-donating activities during migration. The net strength of the interaction and the separation characteristics can be controlled by the PEG concentration. The hydrogen-bonding interaction would be enhanced in hydrophobic circumstances such as a micelle core compared with an aqueous core. With this in mind, we have attempted to effect hydrogen-bonding interactions to assist in MEKC separations using mixed micelle composed of SDS and non-ionic surfactants, such as Brij 35 and Tween 20, having polyether moieties.

## 2. Experimental

Brij 35 [polyoxyethylene(23) lauryl ether], Tween 20 (polyoxyethylene sorbitan monolaurate) and SDS were purchased from Wako (Osaka, Japan), Kishida Chemical (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively, and used as received. Aniline ($NH_2$-Ph), phenol (HO-Ph), acetanilide ($CH_3CONH$-Ph), benzaldehyde (CHO-Ph), phenyl acetate ($CH_3COO$-Ph), *p*-toluidine ($4NH_2$-To), *p*-cresol (4HO-To), *p*-acetotoluidine ($4CH_3COHN$-To), *p*-tolualdehyde (4CHO-To) and *p*-cresol acetate ($4CH_3COO$-To) as model analytes were obtained from Wako, Tokyo Kasei Kogyo (Tokyo, Japan) and Nacalai Tesque. Oil Yellow AB (1-phenylazo-2-naphthylamine) (Nacalai Tesque) was used as a marker of the micelle migration. All other chemicals were of analytical-reagent grade.

Electrophoretic separations were performed using a Jasco (Tokyo, Japan) CE-800 system coupled to a Jasco 807-IT integrator. A 500 × 0.05 mm I.D. capillary was supplied by Jasco; the effective length for separation was 300 mm. As electrolytic solutions, 10 m*M* phosphate buffer (pH 7.8) or 100 m*M* Tris–100 m*M* borate (pH 8.2) were used. Prior to electrophoresis, the capillary was rinsed with 0.1 *M* sodium hydroxide for 10 min, water for 5 min and then with the electrolysis solution for 5 min using an aspirator. Between repeated analysis, the capillary was washed with the electrolysis solution for 5 min. Samples were introduced by siphoning at a height of 15 cm for 5–10 s. The detection of the analytes was performed by UV spectrophotometry at 210 nm.

A series of $^1H$ NMR measurements of the mixed surfactant micellar systems were carried out in $D_2O$ (Euriso-top CEA Group, Saint Aubin, Cedex, France) with a JEOL (Tokyo, Japan) GX-270 instrument operating at 270 MHz using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (Nacalai Tesque) as a reference.

## 3. Results and discussion

### 3.1. Electropherograms in mixed micellar EKC

Fig. 1A, shows an electropherogram of the ten mono- and disubstituted benzenes in the SDS–MEKC mode in the absence of non-ionic surfactant in phosphate buffer ([SDS] = 50 m*M*). The elution order essentially reflects the hydropho-

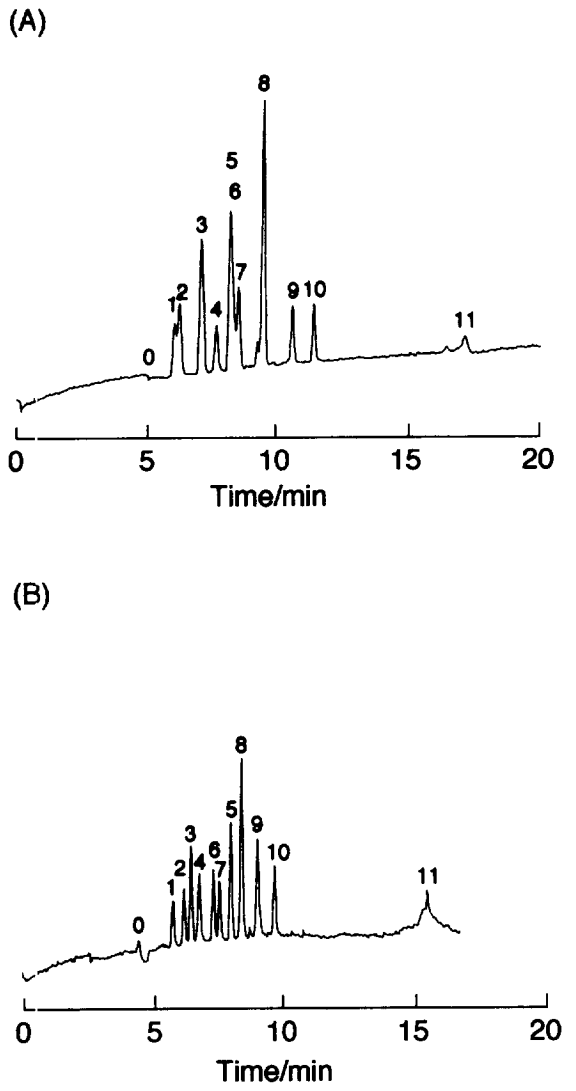


Fig. 1. Micellar EKC separation of ten mono- and disubstituted benzenes in (A) the absence and (B) the presence of 3.5 m*M* Tween 20 in electrolyte solution containing 50 m*M* SDS. Conditions: applied voltage, 10 kV; operating current, 10 $\mu$A; capillary length, 50 cm (effective length 30 cm); electrolyte solution 10 m*M* phosphate buffer containing surfactants (pH 7.8). Peaks: 0 = methanol (a marker of electroosmotic flow); 1 = $NH_2$-Ph; 2 = HO-Ph; 3 = $CH_3CONH$-Ph; 4 = CHO-Ph; 5 = 4HO-To; 6 = $CH_3COO$-Ph; 7 = $4NH_2$-To; 8 = $4CH_3CONH$-To; 9 = 4CHO-To; 10 = $4CH_3COO$-To; 11 = Oil Yellow AB (a marker of micelle migration).

bicity of the analytes. Under these conditions, the separations between $NH_2$-Ph (peak 1) and HO-Ph (peak 2) and that between 4HO-To (peak 5), $CH_3COO$-Ph (peak 6) and $4NH_2$-To (peak 7) were not complete. Among these incompletely separated analytes, HO-Ph, 4HO-To, $NH_2$-Ph, and $4NH_2$-To can inherently act as hydrogen-bonding donors.

Based on our previous CZE experiments using polyethers [20,21], these hydrogen-donating analytes are expected to interact attractively with polyether segments. Thus, Tween 20 or Brij 35 was added at concentrations ranging from 1 to 50 m*M* to the electrolyte solution containing 50 m*M* of SDS, where the non-ionic surfactants and SDS are considered to form a mixed micelle (see later). The addition of Tween 20 provides a significant change in the electrophoretic behaviour. Fig. 1B shows an electropherogram of the model analytes in the MEKC mode in the presence of 3.5 m*M* Tween 20 and 50 m*M* SDS. Under these conditions, almost complete baseline separation was achieved. The greatest effect of the addition of Tween 20 is a relative increase in the migration time of the phenolic compounds (HO-Ph and 4HO-To) compared with those of the other analytes, resulting from the specific enhancement of the transfer of the phenolic compounds into the negatively charged mixed micelle. This is reasonably considered to occur through electrostatic interaction, probably hydrogen-bonding interaction, between the hydroxyl groups of the two analytes and the polyether segments of the non-ionic surfactant in the mixed micelle.

A similar effect was observed using the SDS–Brij 35 system in Tris–borate buffer and the reproducibility of the migration was superior to that of the SDS–Tween 20 system in phosphate buffer. However, the use of Tris–borate buffer for the SDS–Brij 35 system resulted in a decline of the peak resolution even in the absence of Brij 35. In phosphate buffer, the SDS–Brij 35 system was not acceptable probably owing to the adsorption of Brij 35 on the inner wall of the capillary. Such adsorption of Brij 35 has been reported and discussed previously [19,22].

It should be noted that enhancement of separation efficiencies would occur in the SDS–Tween 20 system in comparison with the SDS system, as reported for some mixed micelle

systems [14,19], and contribute to the improvement of separations as seen in Fig. 1B.

### 3.2. Thermodynamic aspects of mixed micellar EKC

We then attempted to quantify the energetic effects of the addition of a non-ionic surfactant on the migration behavior. In MEKC, solutes (analytes) are reasonably considered to be dissolved in equilibrium between aqueous (Aq) and micellar phases (M). The chemical potential of the analyte in the aqueous phase ($\mu_{Aq}$) is expressed as

$$\mu_{Aq} = \mu^{\circ}_{Aq} + RT \ln X_{Aq} \quad (1)$$

where $X_{Aq}$ is the concentration of the solute in mole fraction units and $\mu^{\circ}_{Aq}$ the standard chemical potential on the unitary scale [23]. Assuming that the solute within the micelle constitutes an ideal solution, one can use the analogue of Eq. 1 for the micellar phase:

$$\mu_{M} = \mu^{\circ}_{M} + RT \ln X_{M} \quad (2)$$

Hence the standard free energy of the transfer of the solute to the micellar interior from the aqueous phase (that is, the difference between the free energy of the interaction with the solvents; $\Delta\mu^{\circ} \equiv \mu^{\circ}_{M} - \mu^{\circ}_{Aq}$) is given by

$$\Delta\mu^{\circ} = -RT \ln(X_{M}/X_{Aq}) \quad (3)$$

Under the conditions where $X_{M}$ and $X_{Aq} \ll 1$, $X_{M}/X_{Aq}$ can be related to the distribution constant ($K$) of the analyte by

$$X_{M}/X_{A} \cong K(C_{water,Aq}/C_{sf,M}) \quad (4)$$

where $C_{water,Aq}$ and $C_{sf,M}$ are the molar concentrations of water in the aqueous phase and of the surfactant in micelles, respectively. At low micellar concentrations, the capacity factor ($k'$) in MEKC can be expressed by

$$k' \cong Kv_{M}(C_{sf} - C_{cmc}) \quad (5)$$

where $v_{M}$, $C_{sf}$ and $C_{cmc}$ represent, respectively, the partial specific volume of the micelle, the analytical concentration of the surfactant and the critical micellization concentration (CMC) of the surfactant system [2]. Combining Eqs. 3–5, the transfer free energy can be related to $k'$ as follows:

$$\Delta\mu^{\circ} = -RT \ln(k'\Phi) \quad (6)$$

where $\Phi$ [$= (C_{water,Aq}/C_{sf,M})/v_{M}(C_{sf} - C_{cmc})$] is a parameter characteristic of the micellar system alone and independent of solutes.

The transfer free energy of (neutral) solutes in the pure SDS micellar system ($\Delta\mu^{\circ}$ in Eq. 6) is predominantly ascribed to the hydrophobic interaction between the analytes and the hydrophobic alkyl chain of SDS and denoted by $\Delta\mu^{\circ}_{HP}$. A thermodynamic model for the mixed micellar system consisting of SDS and non-ionic surfactant is proposed as follows. The hydrogen-bonding interaction is considered to work additively with the hydrophobic interaction. At low mixing ratios of non-ionic surfactant, it is assumed that the hydrophobic interaction is virtually independent of the analytical concentration of the non-ionic surfactant ($C_{n}$) and that the additional stabilization energy caused by the incorporation of the non-ionic surfactant is proportional to the concentration of the non-ionic surfactant molecules in the mixed micelle ($C_{n,M}$). Therefore, the transfer free energy in the mixed micellar system ($\Delta\mu^{\circ}_{MM}$) can be expressed by

$$\Delta\mu^{\circ}_{MM} = \Delta\mu^{\circ}_{HP} - kfC_{n} \quad (7)$$

where $k$ is a constant representing the stabilization energy per unit concentration of the non-ionic surfactants in the mixed micelle and $f$ is a condensation factor given by $f = C_{n,M}/C_{n}$. Substitution of $\Delta\mu^{\circ}_{MM}$ in Eq. 7 into $\Delta\mu^{\circ}$ of Eq. 6 yields the following equation describing the relationship between $k'$ and $C_{n}$:

$$RT \ln k' = kfC_{n} - \Delta\mu^{\circ}_{HP} - RT \ln \Phi \quad (8)$$

In this equation, $\Phi$ is also a function of $C_{n}$. In order to eliminate the $C_{n}$ dependence of $\Phi$, a relative value of $k'$ of a given analyte against that of a certain reference compound ($k'_{0}$) will be introduced, since $\Phi$ is independent of solutes as described above. This yields

$$RT \ln(k'/k'_0) = (k - k_0)fC_n - (\Delta\mu^\circ_{HP} - \Delta\mu^\circ_{HP,0}) \quad (9)$$

where $k'_0$ and $\Delta\mu^\circ_{HP,0}$ denote the corresponding values of the reference compound.

Fig. 2 shows the dependence of $RT \ln(k'/k'_0)$ on the analytical concentration of Brij 35 ([Brij 35]), where CHO-Ph was selected as a reference compound because the interaction of CHO group with PEG appears to be negligible in CZE mode [20,21]. The capacity factors were evaluated according to the relationship

$$k' = (t_R - t_0)/[t_0(1 - t_R/t_{mc})] \quad (10)$$

where $t_R$, $t_0$ and $t_{mc}$ are the migration time of the analyte, water and the micelle, respectively [1,2]. The values of $RT \ln(k'/k'_0)$ varies linearly with [Brij 35] at [Brij 35] < 5 m*M* (correlation coefficients $r^2 = 0.993$–$0.958$). The linear relationship is well described by Eq. 9. The slope gives the $(k - k_0)f$ value, which reflects a relative magnitude of the additional interaction with Brij 35 compared with CHO-Ph, while the intercept represents the difference in the hydrophobic stabilization energy in the pure SDS micellar system between a given analyte and CHO-Ph $(\Delta\mu^\circ_{HP,0} - \Delta\mu^\circ_{HP})$. Table 1 summarizes the $(k - k_0)f$ values thus evaluated for the ten substituted benzenes. The phenolic compounds (HO-Ph and 4HO-To) with strong hydrogen-donating activity yielded the largest values of $(k - k_0)f$ over 200 kJ l mol$^{-2}$, indicating the strong attractive interaction with Brij 35. The other hydrogen-donating active analytes with the amino or amide group ($NH_2$-Ph, $4NH_2$-To, $CH_3CONH$-Ph, $4CH_3CONH$-To) also gave positive values of $(k - k_0)f$. In contrast, the $(k - k_0)f$ values of the hydrogen-donating inactive analytes ($CH_3COO$-Ph, $4CH_3COO$-To and 4CHO-To) were negative. These results are fundamentally described in terms of the hydrogen-bonding interaction. The best separation was achieved at [Brij 35] = 2 m*M* as judged from Fig. 2. In the case of the SDS–Tween 20 system also, similar relationships between $RT \ln(k'/k'_0)$ and [Tween 20] were obtained, in which the best resolution was observed at [Tween 20] = 3.5 m*M* (Fig. 1).

Now consider the energetic contribution of the methyl substituent to the hydrophobic and hydrogen-bonding interactions. The intercepts in Fig. 2 (at [Brij 35] = 0) of the toluene derivatives (HO-To, $NH_2$-To, $CH_3CONH$-To, CHO-To and $CH_3COO$-To) are larger than those of the corresponding monosubstituted benzenes (HO-Ph,

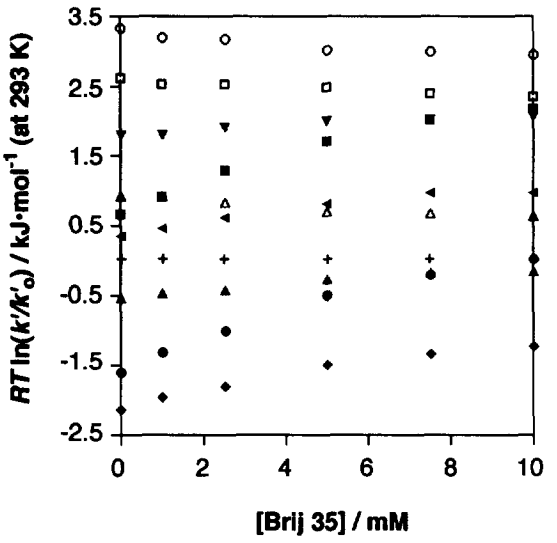


Fig. 2. Dependence of $RT \ln(k'/k'_0)$ of the substituted benzenes on the concentration. Conditions: voltage, 10 kV; operating current, <23 μA; capillary length, 50 cm (effective length 30 cm); electrolyte solution, 100 m*M* borate–100 m*M* Tris buffer containing surfactants (pH 8.2). ● = HO-Ph; ■ = 4HO-To; ◆ = $NH_2$-Ph; ◀ = $4NH_2$-To; ▲ = $CH_3CONH$-Ph; ▼ = $4CH_3CONH$-To; △ = $CH_3COO$-Ph; ○ = $4CH_3COO$-To; □ = 4CHO-To; + = CHO-Ph (as a reference compound).

Table 1
Slopes of $RT \ln(k'/k'_0)$ vs. [Brij 35] plots at 293 K

| Compound | Slope (kJ l mol$^{-2}$) |
|---|---|
| HO-Ph (phenol) | 218 |
| 4HO-To (*p*-cresol) | 210 |
| $NH_2$-Ph (aniline) | 124 |
| $4NH_2$-To (*p*-toluidine) | 93 |
| $CH_3CONH$-Ph (acetanilide) | 52 |
| $4CH_3CONH$-To (*p*-acetotoluidine) | 42 |
| CHO-Ph (benzaldehyde) | 0 |
| 4CHO-To (*p*-tolualdehyde) | −24 |
| $CH_3COO$-Ph (phenyl acetate) | −47 |
| $4CH_3COO$-To (*p*-cresol acetate) | −54 |

Conditions as in Fig. 2.

$NH_2$-Ph, $CH_3CONH$-Ph, CHO-Ph and $CH_3COO$-Ph) by about 2.4 kJ $mol^{-1}$, which corresponds to the hydrophobic stabilization energy associated with the transfer of the methyl group from aqueous to the SDS micelle ($-\Delta\mu^{\circ}_{HP,Me}$). This stabilization energy is smaller than that of the methyl group of linear hydrocarbons in SDS micellar system (ca. 4 kJ $mol^{-1}$) [23]. The discrepancy might be ascribed to the difference in the environments of the methyl groups attached to the aromatic carbon and the linear aliphatic carbon.

On the other hand, the slopes in Fig. 2 [i.e., the $(k - k_0)f$ values in Table 1] of the toluene derivatives (HO-To, $NH_2$-To, $CH_3CONH$-To, CHO-To and $CH_3COO$-To) are almost the same as those of the corresponding monosubstituted benzenes (HO-Ph, $NH_2$-Ph, $CH_3CONH$-Ph, CHO-Ph and $CH_3COO$-Ph). This result indicates that the methyl group which is incapable of hydrogen-bonding complex formation does not participate in the specific interaction with Brij 35 and also that the hydrophobic interaction remains almost unchanged during the addition of Brij 35. This is all in agreement with our thermodynamic model described above.

The energetic effect of the hydrogen-bonding interaction between the phenols and Brij 35 may be estimated as follows. The volume ratio of the aqueous and pseudo-stationary phases in our SDS–MEKC $\{=[v_M(C_{sf} - C_{cmc})]^{-1}$ in Eq. 5$\}$ can be evaluated as 140 using $k' = 0.522$ for HO-Ph at [SDS] = 50 m$M$ and $K = 73$ for HO-Ph in the pure SDS micellar system [2]. Assuming that all of Brij 35 molecules are incorporated into the mixed micelle (that is, $f \approx 140$), values of $k - k_0$ for the phenols are evaluated at about 1.5 kJ l $mol^{-2}$. For an imaginary pure solution of Brij 35, the molar concentration might be about 0.8 $M$ ($M_r$ 1214, $d \approx 1$). Therefore, the hydrogen-bonding interaction energy between the phenols and Brij 35 would be estimated to be about 1.2 kJ $mol^{-1}$. The energy thus evaluated seems to be smaller than that of the usual hydrogen bonding. This would be mainly due to the weak hydrogen-accepting ability of Brij 35. However, the energy is sufficient to improve the selectivity in MEKC as described above.

All the behaviour described above appear to be fundamentally in accord with our thermodynamic model. However, one discrepancy is that the $(k - k_0)f$ values of $NH_2$-Ph and $4NH_2$-To are larger than those of $CH_3CONH$-Ph and $4CH_3CONH$-To, in spite of the fact that the amide group is a stronger hydrogen donor than the amino group. Similar disagreement is observed in the comparison of the $(k - k_0)f$ values between $CH_3COO$-Ph and CHO-Ph, and between $4CH_3COO$-To and 4CHO-Ph: neither acetoxy nor formyl groups act as hydrogen donors, but the $(k - k_0)f$ values of $CH_3COO$-Ph and $4CH_3COO$-To are more negative than those of CHO-Ph and 4CHO-Ph. These phenomena are in contrast with those in the CZE mode using PEG as a matrix, in which the order of the interaction of the substituents with PEG is OH > CONH > $NH_2 \gg CH_3COO \geq$ CHO [20,21]. The cause of this discrepancy is not clear. Some other interaction might be operative in this mixed micellar EKC mode.

Very recently, Quina et al. [24] reported a liner solvation free energy relation analysis for the transfer of non-ionic solutes into various micelles, in which a considerable positive dependence of the transfer energy on the hydrogen-bonding acidity of solutes was evidenced in the case of Brij 35 micelles compared with SDS micelle. This supports well our arguments with respect to hydrogen-bonding interaction in the Brij 35-containing mixed micelle.

### 3.3. Formation of mixed micelles

The CMC values of non-ionic surfactants are generally much lower than those of ionic surfactants as long as they have the same alkyl chain length. Therefore, almost all molecules of Tween 20 and Brij 35 would be considered to form mixed micelles with SDS molecules. That mixed micelle formation occurs can be supported by the following experimental results. The first concerns the EKC behaviour. The $k'$ values of the analytes with the strong hydrogen-donating activity, such as HO-Ph and 4HO-To, increased selectively and remarkably with an increase in [Tween 20] or [Brij 35] up to about 15 m$M$ under our experimental conditions. Considering the relatively strong attractive interaction between these

analytes and the polyether moieties of Tween 20 or Brij 35, the non-ionic surfactant-containing micelles which interact with the analytes should have negative charges in order to reduce the relative migration velocities of the analytes compared with the velocities of the other analytes (note here that the detection was performed at the negative potential side). This obviously indicates the formation of mixed micelles with SDS. The second is NMR spectroscopic evidence. The $^1$H signal on the C-1 and the C-2 carbon atoms of SDS shifted to a slightly higher magnetic field with increasing [Tween 20] or [Brij 35] in the mixed surfactant systems. These shifts were ca. −0.020 ppm for $^1$H on C-1 and ca −0.015 ppm for $^1$H on C-2 at [SDS] = 50 m*M* and [Brij 35] or [Tween 20] = 10 m*M*. This indicates a change in the electrostatic environment near the sulfonic group of SDS, probably owing to the interaction of the sulfonic group with the polyether moieties of the non-ionic surfactants. Since the concentration of free Tween 20 or Brij 35 was kept very low as described above, the $^1$H signal shifts are reasonably ascribed to the formation of mixed micelles. In contrast, the $^1$H signal on the C-12 carbon atom (the methyl group) of SDS remained almost unchanged. This may indicate that the hydrophobic environment in the micellar core is scarcely affected by the addition of the non-ionic surfactants.

It is important to note that the addition of the non-ionic surfactants provides a significant change in the electrophoretic velocity of the mixed micelle ($V_{ep,mc}$). In this study, values of $V_{ep,mc}$ were estimated on the basis of their relationship with the net (measured) migration velocity of the micelle ($V_{mc}$):

$$V_{mc} = V_{ep,mc} + V_{eo} \quad (11)$$

where $V_{eo}$ is the electroosmotic velocity with the sign (plus) opposite to that of $V_{ep,mc}$ for the negatively charged micelles. The value of $V_{eo}$ was evaluated from the migration time of methanol, which was co-injected with the samples. Fig. 3 shows $V_{ep,mc}$ values as a function of [Brij 35] or [Tween 20]. The absolute values of $V_{ep,mc}$ decrease with increasing [Brij 35] or [Tween 20] in the concentration range 1–15 m*M*. This seems to

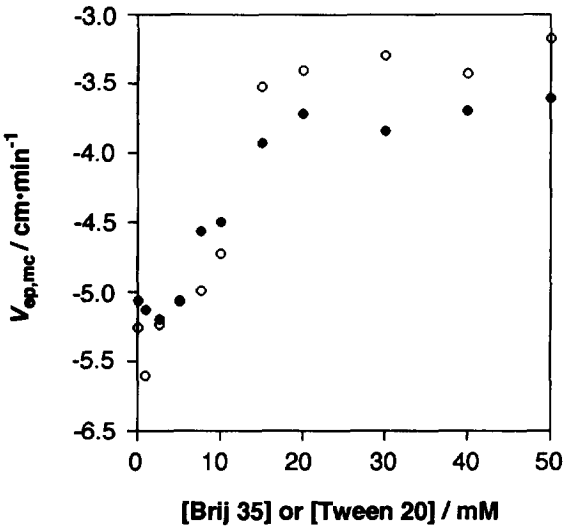


Fig. 3. Electrophoretic velocity of the mixed micelles as a function of the concentration of (○) Brij 35 or (●) Tween 20. Conditions as in Figs. 1 and 2.

suggest an increase in the hydrodynamic radius of the micelles with the addition of the non-ionic surfactants. Such a decrease in $V_{ep,mc}$ for the SDS–Brij 35 system has been reported [19].

At [Brij 35] or [Tween 20] over 15 m*M*, however, $V_{ep,mc}$ became almost independent of [Brij 35] or [Tween 20] or decreased very slightly with increasing [Brij 35] or [Tween 20] (Fig. 3). Under these conditions, the capacity factor ($k'$) of all the analytes examined decreased considerably with increase in [Brij 35] or [Tween 20] (data not shown). These phenomena suggest the occurrence of drastic change in structure of the mixed micelle. Anyway, such a drastic decrease in $k'$ values of all analytes results in poor separation of analytes. Hence the practically available concentration of Brij 35 and Tween 20 would be less than 15 m*M* at least at [SDS] = 50 m*M*. Interestingly, such phenomena were not observed when Brij 30 was used instead of Brij 35 in the concentration range 1–100 m*M*. Brij 30 has only four ethylene glycol segments, whereas Brij 35 has 23 such segments. Therefore, the relatively long polyether chains of Brij 35 or Tween 20 appear to be responsible for the occurrence of the drastic change in the structure of the mixed

micelles [25]. Detailed studies of these phenomena are in progress.

In conclusion, the incorporation of non-ionic surfactants with polyether moieties into the SDS system to form mixed micelles is found to provide a pseudo-stationary phase possessing valuable selectivity. Hydrogen-bonding interactions between the analytes and the polyether moieties appear to work additively with the hydrophobic interaction in the mixed micellar EKC, resulting in an improvement of the separation. The hydrogen-bonding interaction in MEKC is expected to be more effective than in the CZE mode, because hydrogen-bonds will form more strongly in the hydrophobic micellar core.

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