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Review

Hydrophilic selectors for selectivity enhancement in capillary electrophoresis

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Abstract

Several hydrophilic selectors useful for improving selectivity in capillary electrophoresis are described. The interaction between the hydrophilic selectors and analytes is basically described in terms of the hydrogen-bonding interaction, which is frequently encountered for organic compounds, especially biological substances. Although the hydrogen-bonding interaction in aqueous media is relatively weak in strength, it is enough to vary the migration patterns of analytes, especially when the hydrophobic interaction works cooperatively. This review highlights polyethers as hydrogen-accepting agents. Thermodynamic aspects of the hydrogen-bonding interaction are also reviewed. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Reviews; Hydrophilic selectors; Buffer composition; Hydrogen bonding

Contents

1. Introduction

The development of various new techniques to control migration in capillary electrophoresis (CE) is important for extending the availability of CE. Since the separation in CE is performed in homogeneous phases, various interaction modes can be easily introduced into the separation systems of CE by adding appropriate agents to the electrolyte solutions. Interactions between analytes and agents cause

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changes in the apparent hydrodynamic sizes and/or apparent charges of the analytes, resulting in variation of the migration patterns, reflecting characteristics of the analytes. Novel selectivity has been realized using many kinds of charged carriers, ion-pairing reagents, complexing reagents and host compounds, such as cyclodextrins (CDs) [1–4]. A deeper knowledge of separation mechanisms is of particular interest for the development of novel separation systems.

The hydrophobic interaction has been utilized most frequently in CE, as obviously recognized in the principle of micellar electrokinetic chromatography (MEKC) [5,6]. The hydrophobic interaction can work effectively in aqueous electrolyte solutions, in which most CE experiments are performed. In contrast, hydrophilic interactions, such as the hydrogen-bonding interaction, will be thermodynamically disadvantageous in aqueous phases compared with non-aqueous surroundings. The formation of hydrogen bonds is frequently encountered for organic compounds, especially biological substances. Such compounds exhibit a variety of hydrogen-bonding activities. Thus, the hydrogen-bonding interaction can be expected to be utilized to improve the CE separation of a wide range of samples. In chiral separation using CDs and micelles, the hydrogen-bonding interaction also seems to play an important role. The hydrophilic moiety of the surfactants used in MEKC is often responsible for characteristic separation selectivity [6,7]. In such cases, the hydrogen-bonding interaction would work with the assistance of the hydrophobic interaction between analytes and additives. The hydrophobic surroundings provided by CDs and micelles would facilitate the hydrogen-bonding interactions, which are responsible for improved selectivity.

Polyethers are known to serve as electrostatic electron donors via the ether oxygen atoms. Well-known examples are inclusion phenomena of cations by crown ethers and non-cyclic polyethers [8,9]. It is likely that such electron donors also serve as hydrogen acceptors. The hydrogen-bonding formation between biological non-cyclic polyether ionophores and amines has been recognized in the complexed crystal [10]. The significance of polyethers has also been pointed out for the improvement of separations [11–14] and chiral separation in CE [15,16].

This report will review the control and improvement of separations by the use of hydrophilic selectors, especially polyether additives. Emphasis is placed on the hydrogen-bonding interactions that are useful for CE separations. Some thermodynamic aspects that are useful for describing the systems and constructing separation strategies using hydrophilic selectors that promote hydrogen-bonding interactions will also be reviewed.

2. Hydrophilic selectors in CE separation

2.1. Capillary zone electrophoresis (CZE) separation

CDs are useful as additives for the separation of structural isomers and structurally related compounds. The successful separation of nine plant growth regulators has been realized using a mixture of α-, β- and γ-CDs as modifiers [17], where the hydrophilic interaction between the hydroxyl groups of CDs and the functional groups of the analytes seems to be responsible for the improved separation, as well as the sizes of the analytes and the cavities of the CDs. A variety of organic and inorganic ions have been separated in isotachophoresis by using CDs as additives [18]. The chiral separation of dansyl-amino acids has been performed by incorporating β-CD in a gel matrix and a model of interaction including hydrogen-bonding between the hydroxyl groups of β-CD and the dansyl groups of the analytes has been proposed [19]. It has been accepted generally that the binding forces involved in complex formation between CDs and guest molecules are hydrophobic interactions between the hydrophobic moieties of the guest molecules and the CD's cavity, hydrogen-bonding between the polar functional groups of guest molecules and the hydroxyl groups of CDs, release of high energy water molecules from the cavity during complex formation and the release of strain energy in the ring frame system of the CDs [18]. Several hydrophilic host compounds, such as CDs, crown ethers and proteins, have been used in chiral CZE separations. The separation of optical isomers of compounds in CE was reviewed in detail recently [15,16]. In all of these cases, the hydrogen-bonding interaction would

play an important role in the separation of a variety of organic compounds possessing hydrogen-bonding activities and the interaction is facilitated by the hydrophobic interaction between analytes and additives.

Poly(ethylene oxide) (PEO) is utilized as a molecular sieving material in the separation of sodium dodecyl sulfate (SDS)–protein complexes [20–24] and DNA fragments [25] and as a coating material to prevent the adsorption of analytes on to the inner walls of the capillary and/or to suppress the electroosmotic flow [26–28]. In such strategies, it is basically thought that PEO does not interact specifically with target analytes. However, the addition of PEO causes a remarkable change in the migration behavior of analytes in some CE separations [11–14], indicating that PEO can interact with analytes even in aqueous media. Poly(ethylene glycol) (PEG), being a PEO agent, is used as a separation matrix in the capillary isotachophoresis of some chlorophenols [11]. It is suggested that chlorophenols are distributed between the PEG polymer network and the aqueous phase. A two-component polymer system with PEO–dextran (PD) as a matrix is effective for the separation of small pharmaceutical compounds [12]. Pure PEO and dextran systems exhibit different selectivities from each other. Therefore, one can easily optimize the separation by simply varying the ratio of these two compounds and their concentrations.

PEG is also used as an additive in the CZE separation of substituted benzoates (PEG-assisted CZE) [13,14]. Fig. 1 shows the CZE separation of nine substituted benzoates in the absence (A) and presence (B) of 7.5% (v/v) of PEG 4000 [29]. A remarkable improvement in the separation is achieved by the addition of PEG. The change in the migration pattern reflects the interaction of the

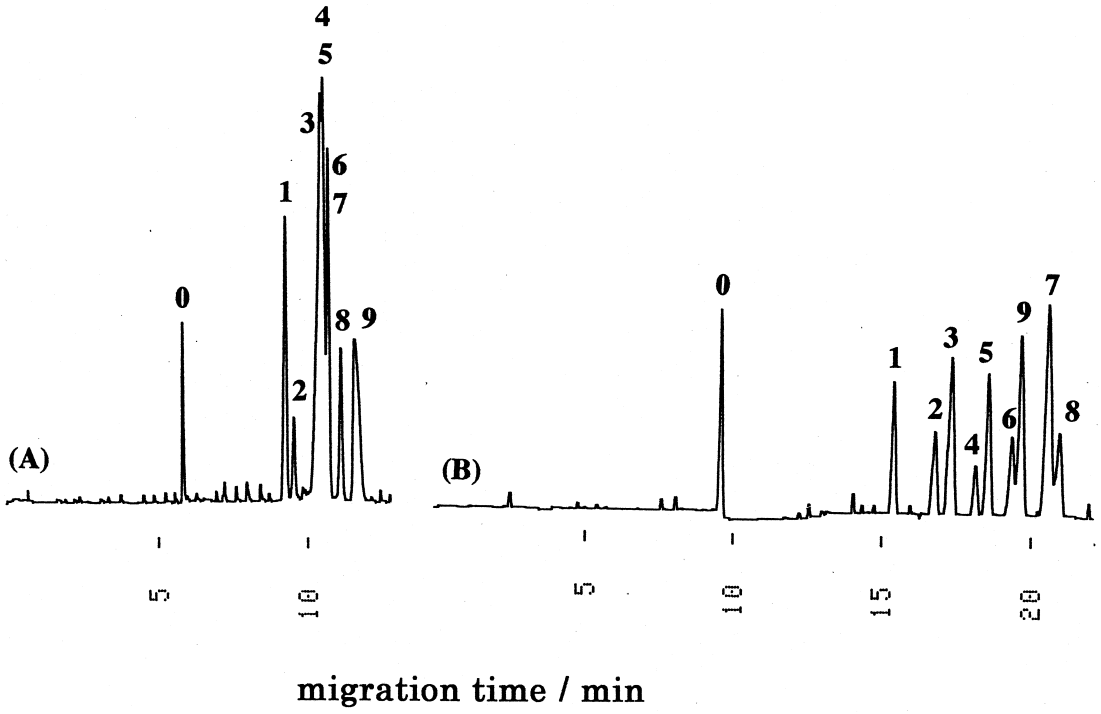

Fig. 1. Electropherograms of nine substituted and unsubstituted benzoic acids in (A) the absence of and (B) the presence of 7.5% (v/v) PEG 4000 in electrolyte solutions. Conditions: Electrolyte solution, 10 m*M* phosphate buffer (pH 7.8); capillary, 750×0.05 mm I.D. (effective length=500 mm); applied voltage (current), 14 kV [7 and 5 μA in (A) and (B), respectively]; detection wavelength, 210 nm. Peaks: (0) mesityl oxide; (1) 4-acetamidobenzoic acid, (2) 4-acetoxybenzoic acid, (3) 4-hydroxybenzoic acid, (4) 4-toluic acid, (5) 4-aminobenzoic acid, (6) telephthalaldehydic acid, (7) 2-phthalaldehydic acid, (8) benzoic acid and (9) salicylic acid.

analytes with PEG as well as a decrease in the electroosmotic flow rate due to the elevated viscosity of the electrolyte solution. PEG is non-ionic and migrates at the electroosmotic flow-rate. The attractive interaction then causes a decrease in the relative migration time. As shown in Fig. 1, analytes with hydrogen-donating substituents, such as CH_3CONH, HO and NH_2 groups (peaks 1, 3, 5 and 9), interact more strongly with PEG than the other non-hydrogen-donating analytes. This indicates that the hydrogen-bonding interaction works effectively in the PEG-assisted CZE, in which the polyether moiety of PEG serves as a hydrogen acceptor (see below for qualitative discussion).

Polyvinylpyrrolidone has been used as a polymeric additive for the CZE separation of diastereomeric derivatives of enantiomers such as D- and L-amino acids. It has been suggested that the selectivity of the system is based on hydrophilic as well as hydrophobic and $\pi-\pi$ and $n-\pi$ interactions between the polymer and the analytes [30–33].

2.2. MEKC separation

MEKC is, in principle, based on the hydrophobic interaction. However, selectivity in MEKC depends, in part, on the kinds of hydrophilic moieties of the surfactants used [6,7]. This suggests that some hydrophilic interactions, including the hydrogen-bonding interaction, contribute to the separation. In chiral separations, the hydrogen-bonding interaction between polar functional groups of analytes and optically active hydrophilic groups of surfactants in micelles seems to work in optical recognition [15,16].

Some bile salt systems were compared with SDS systems regarding the selectivity of MEKC [34,35]. Fourteen ingredients in a cold medicine have been successfully separated on a sodium cholate (SC) system and on a sodium deoxycholate system. The two migration patterns are remarkably different from that on the SDS system. This would be ascribed mainly to the difference in the micellar structure. However, several obvious differences are also observed between the cholate and deoxycholate systems [34]. Bile salts have a variety of hydroxyl groups, both in position and number. Therefore, some hydrophilic interactions between the hydroxyl groups and analytes seem to be responsible for the differences in the migration pattern.

Disodium 5,12-bis(dodecyloxymethyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulphonate, which has six ether oxygen atoms, is used in the MEKC separation of substituted benzenes and substituted naphthalenes. Considerably different selectivities are observed compared with those obtained using SDS [36]. Twenty three dansyl-amino acids and short peptides have been successfully separated on a micelle system consisting of non-ionic Tween 20, which possesses polyether chains as hydrophilic groups [37,38]. Polyethers can work as hydrogen acceptors and then the hydrogen-bonding interaction as well as the hydrophobic interaction should play an important role in the separation of these analytes.

Fig. 2 shows the MEKC separation of four substituted benzenes using three surfactant systems: SDS, SC and lithium perfluorooctanesulfonate (LiPFOS) [39]. The migration patterns are completely different from each other. In particular, the order of the migration of analytes in the LiPFOS system is the reverse of that found in the SC system. The phenomena could not be simply interpreted in terms of the hydrophobic interactions. This result suggests the significance of the hydrogen-bonding interaction (see below).

Mixed surfactant systems are frequently used in MEKC. The advantages of mixed surfactant systems is an increase in the number of optimizing system parameters, which involves characteristics of surfactants and the mixing ratio. The successful separation of seventeen corticosteroids has been achieved in mixed surfactant systems composed of bile salts and SDS [40]. The addition of SDS to the bile salt micelle broadens the elution window of the MEKC system, resulting in drastic changes in selectivity and an improvement in the resolution.

Non-ionic surfactants, such as the Tween- and Brij series, are also available for the separation of non-ionic analytes, by constructing mixed micelles with ionic surfactants. Mixed surfactant systems, consisting of these non-ionic surfactants and SDS, have exhibited beneficial changes in selectivity in the separation of substituted benzenes as model samples [41–45] and the baseline resolution of ten substituted benzenes has been achieved [45]. The Tween- and

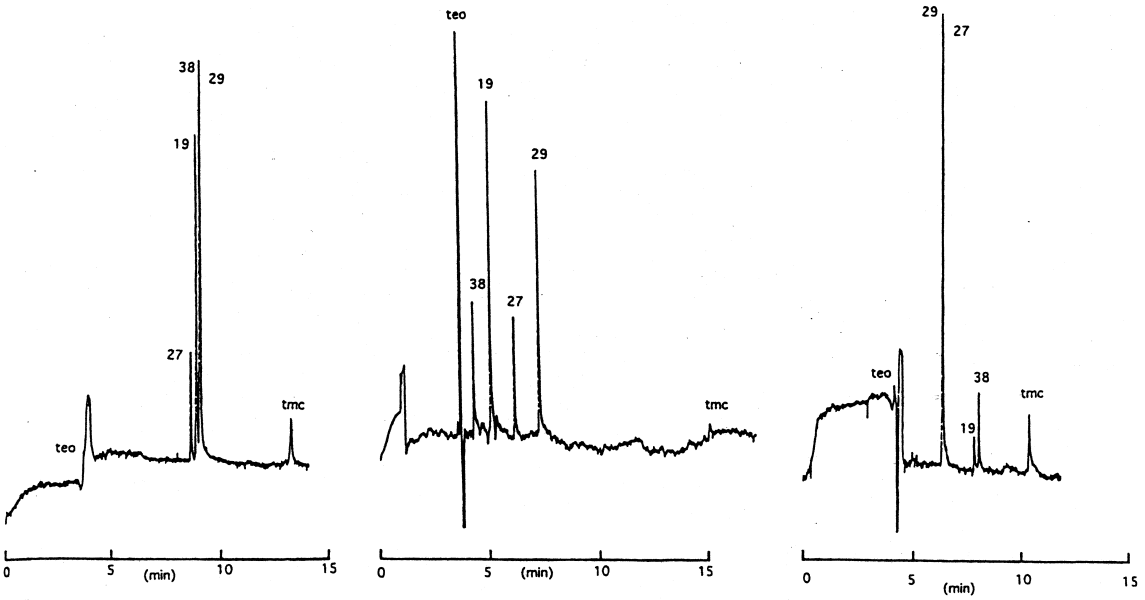

Fig. 2. MEKC elution patterns for (a, left) 40 m*M* SDS, with UV detection at $\lambda = 210$ nm, (b, middle) 40 m*M* LiPFOS, with UV detection at $\lambda = 214$ nm and (c, right) 80 m*M* SC, with UV detection at $\lambda = 254$ nm. Experimental conditions: 50 m*M* phosphate, pH 7.0, 20 kV. Peaks: (19) bromobenzene, (27) 4-bromonitrobenzene, (29) 4-chloroacetophenone and (38) 4-iodophenol. (from ref. [39]).

Brij series have polyether chains as a hydrophilic moiety and the hydrogen-bonding interaction between the polyether chain of the surfactants and hydrogen-donating analytes is responsible for the change in the migration patterns of the analytes [45]. A Brij 35–SDS system has been used to improve the separation in the separation of herbicides [46]. Adrenaline and its precursors (as cationic analytes) have also been successfully separated on a Tween 20–SDS system [47], and the contribution of hydrogen-bonding interactions to the separation can be seen in this case.

CDs are also added to micellar solutions and employed in the separation of highly lipophilic analytes and in the separation of optical isomers of compounds. In this CD–MEKC system, CDs compete with micelles to bind to analytes. This results in an improvement in the separation of highly lipophilic compounds that are difficult to separate from each other using pure SDS systems because they are predominantly dissolved in the SDS micelles [48,49]. On the other hand, in chiral separations, CDs serve as chiral selectors as in CZE separations [15,16].

2.3. PEG-assisted CZE systems

The interaction of PEG with analytes in PEG-assisted CZE has been quantitatively expressed on the basis of a model of formation of a stoichiometric hydrogen-bonding complex between an analyte and PEG [13,14]. The observed electrophoretic velocity of the analyte (V_{ep}) is expressed as a function of the PEG concentration ([PEG]) by Eq. (1),

$$\frac{V_{ep}}{V_{ep,0}} = \frac{V_{ep,f}}{V_{ep}} + K\left[\frac{V_{ep,c} - V_{ep,f}}{V_{ep,0}}\right][\mathrm{PEG}] \qquad (1)$$

where K is the hydrogen-bonding complex formation constant and $V_{ep,f}$ and $V_{ep,c}$ are the electrophoretic velocities of the free analyte and the analyte–PEG complex, respectively. Because the change in [PEG] affects the viscosity of the electrophoretic medium and, therefore, the electrophoretic velocity, the viscosity effect on the electrophoretic velocity is eliminated by expressing the electrophoretic velocity as a relative value against that of a reference compound with $K \approx 0$ ($V_{ep,0}$). For such ionic analytes, a value of

$V_{ep,c} - V_{ep,f}$ is reasonably assumed to be $-V_{ep,f}$, since PEG is neutral with a sufficiently large hydrodynamic size. Linear relationships between $V_{ep}/V_{ep,0}$ and [PEG] have been verified experimentally for benzoate derivatives at low concentrations of PEG [13,14]. Thus, values of K can be evaluated from the slopes and intercepts of the linear plots of $V_{ep}/V_{ep,0}$ against [PEG].

Estimated K values of the substituted benzoates depicted in Fig. 1 are summarized in Table 1 for PEG 400, PEG 4000 and PEG 20 000, where the 2-phthalaldehydic acid anion was selected as a reference compound [29]. The K values of substituted benzoates with hydrogen-donating substituents (HO, CH_3CONH and NH_2 groups) are larger than those of non-hydrogen-donating analytes. The order of magnitude of the interactions for the hydrogen-donating substituents ($OH \geq CH_3CONH > NH_2$) is consistent with hydrogen-donating activity. The result can be fundamentally interpreted in terms of the hydrogen-bonding interactions between PEG and the hydrogen-donating analytes.

The effects of the capillary's temperature and the concentration of urea added as a hydrogen-bonding competitor on the K values and also NMR studies have clearly supported the view that the interactions between PEG and the hydrogen-donating analytes are predominantly governed by the hydrogen-bonding interactions [14]. Using the Van't Hoff equation: $d \ln K/d(1/T) = -\Delta H/R$, a ΔH value for 4-hydoxybenzoate was estimated as -1.2 kcal mol^{-1} [29] (1 cal = 4.184 J). The value might be somewhat smaller than that of usual hydrogen-bonding, but it would be reasonable, taking account of the fact that this ΔH value represents a difference between the complex formation energy with PEG and the (competitive) solvation energy in the aqueous phase.

Table 1
Estimated values of complex formation constants (K)

Compounds	$K \cdot 10^3$ ([PEG]$^{-1}$ [a])		
	PEG 400	PEG 4000	PEG 20 000
4HO-BA	10.0	19.4	21.5
$4CH_3CONH$-BA	9.5	19.1	22.4
2HO-BA	9.7	16.7	21.0
$4NH_2$-BA	6.6	10.9	12.7
$4CH_3$-BA	7.0	12.8	14.3
$4CH_3COO$-BA	4.8	9.4	11.9
4CHO-BA	4.5	7.2	7.9
BA	2.7	4.6	5.9
2CHO-BA[b]	0	0	0

[a] [PEG] in % (v/v).
[b] 2CHO-BA is the reference compound, the K value of which is defined as zero.

Even in PEG-assisted CZE systems, the hydrophobic interaction due to the inherent hydrophobicity of PEG and analytes plays an important role. The relatively large K value of the 4-toluic acid anion (4-methyl benzoate) (Table 1) is ascribed to the hydrophobic interaction with PEG. In addition, the hydrophobic interaction would assist the hydrogen-donating analytes in approaching PEG and the hydrophobic surroundings provided by PEG would facilitate the hydrogen-bonding interactions with relatively strong orientation.

Since the stabilization energy of hydrogen-bonding with PEG in an aqueous phase is relatively low, the large hydrodynamic molecular size of PEG is an important factor in PEG-assisted CZE. In other words, the large difference in the hydrodynamic size upon the weak complex formation with PEG is responsible for a remarkable change in migration patterns. When some small (neutral) molecules are used as additives in CZE in the hydrogen-bonding interaction mode, the value of $V_{ep,c} - V_{ep,f}$ in Eq. (1) approaches zero, where practically no improvement is expected in the separation, even if K is large. On the other hand, the K values depend on the molecular mass of PEG also, as shown in Table 1: The estimated K values of a given analyte increase with an increase in the molecular mass of PEG. This might be ascribed to some underestimation of K for relatively low-molecular-mass PEGs, due to the assumption that $V_{ep,c} - V_{ep,f} \approx -V_{ep,f}$. An alternative interpretation, however, of a characteristic migration model along the PEG chain has been proposed [13].

2.4. MEKC systems

2.4.1. Linear solvation free energy relationships (LSER) methodology

The solute–micelle interaction in MEKC has been quantitatively characterized on the basis of LSER methodology [50]. LSER expresses a solubility-related property (SP) concerning the solute–solvent interactions, as given by Eq. (2):

$$\log SP = c + a\Sigma\alpha_2 + b\Sigma\beta_2 + s\pi_2 + rR_2 + v(V_x/100) \quad (2)$$

where $\Sigma\alpha_2$, $\Sigma\beta_2$, π_2, R_2 and V_x are medium-independent parameters of solutes and represent the hydrogen-bonding acidity, hydrogen-bonding basicity, dipolarity, excess molar refraction index and the volume of solutes, respectively. Their relative contributions are dictated by the system's coefficients: *a*, *b*, *s*, *r* and *v*, respectively. The parameter, SP, is related to the partitioning coefficients, capacity factors and their analogues, depending on the partitioning system used, the conditions of which (such as the phase ratio) are expressed by the constant *c* alone. Therefore, a direct comparison of the coefficients *a*, *b*, *s*, *r* and *v* is allowed for the characterization of micelles between the different systems. Estimation of the system's coefficients (*a*, *b*, *s*, *r* and *v*) has been performed by multiple linear regression analysis based on the LSER model using a number of solutes of which $\Sigma\alpha_2$, $\Sigma\beta_2$, π_2, R_2 and V_x values are known.

Table 2 summarizes the evaluated system coefficients for eight surfactants: SDS, SC, LiPFOS, dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (C_{14}TAB), cethyltrimethylammonium bromide (CTAB), Elvacite and Brij 35 [39,51–53]. The contributions of the hydrogen-bonding interactions appear in the coefficients *a* and *b*, which represent the hydrogen-bonding (acceptor) basicity and hydrogen-bonding (donor) acidity, respectively. As judged from Table 2, SC has the largest *a* value and the smallest *b* value among the anionic surfactants (SDS, SC and LiPFOS), reflecting the hydrogen-acceptor-like characteristics, while LiPFOS, having the smallest *a* value and the largest *b* value, may serve as a hydrogen donor. The opposite characteristics of SC and LiPFOS are responsible for the reversed order of the migration in the SC- and LiPFOS-based MEKC systems for the analytes given in Fig. 2. As a hydrogen donor with the largest $\Sigma\alpha_2$ value (0.71) of the analytes used, 4-iodophenol interacts strongly with SC (resulting in an increase in the migration time), while 4-chloroacetophenone, as a hydrogen acceptor with the largest $\Sigma\beta_2$ value (0.45), interacts strongly with LiPFOS. On the other hand, Brij 35, with the polyether chain as a hydrophilic moiety, has a much larger value of *a* than SC. This means that Brij 35 and other polyether compounds serve as efficient hydrogen-bonding acceptors. Although non-ionic Brij and/or Tween series cannot be used alone for the MEKC separation of non-ionic analytes, their strong ability as hydrogen acceptors can be utilized in mixed micelle MEKC.

2.4.2. Mixed surfactant systems

The significance of the addition of a non-ionic surfactant to a pure SDS system has been clearly demonstrated as changes in the capacity factors of analytes [45]. Assuming that the additional interaction energy related to the non-ionic surfactant is proportional to the analytical concentration of the

Table 2
Published LSER coefficients for various surfactants

Surfactant	SP	*c*	*a*	*b*	*s*	*r*	*v*	*n*[a]	ρ[b]	Ref.
SDS (40 m*M*)	k'	−1.49	−0.18	−1.80	−0.26	–	3.95	60	0.9553	[39]
SDS	K_s^c	−0.62	−0.08	−1.84	−0.57	0.32	3.25	66	0.9895	[53]
SC (60 m*M*)	k'	−1.62	0.23	−2.88	−0.27	–	3.89	60	0.9684	[39]
LiPFOS (40 m*M*)	k'	−1.51	−0.98	0.16	−0.25	–	2.44	60	0.9511	[39]
DTAB	K_s^c	−0.87	0.28	−1.82	−0.40	0.57	2.98	39	0.975	[53]
C_{14}TAB (10 m*M*)	k'	−1.78	0.99	−2.75	−0.26	–	3.96	60	0.9578	[52]
CTAB	K_s^c	−0.76	1.02	−3.78	−0.32	0.76	3.57	42	0.986	[53]
Elvacite 2669 (2%)	k'	−1.55	0.24	−2.33	0.09	–	3.00	60	0.9543	[52]
Brij 35	K_s^c	−1.39	1.62	−3.83	−0.37	1.63	3.65	19	0.99	[53]

[a] *n* is the number of test solutes.
[b] ρ is the correlation of linear regression.
[c] K_s is the pseudophase incorporation coefficient.
The relationship between K_s, k' and *P* (partitioning coefficient) is $k' = vK_s = Pv(C_{sf} - \mathrm{CMC})$, where *v*, C_{sf} and CMC are the molar volume of surfactant, the surfactant's analytical concentration and the critical micelle concentration, respectively.

non-ionic surfactant (C_n), at low C_n, the transfer free energy of a solute in a mixed surfactant system ($\Delta\mu^o_M$) can be written as Eq. (3)

$$\Delta\mu^o_M = \Delta\mu^o_{SDS} + kC_n \quad (3)$$

where k is the proportional constant representing the additional energy related to the non-ionic surfactant. On the other hand, the capacity factor (k') in MEKC for an analyte is related to the transfer free energy ($\Delta\mu^o$) in a micellar solution, as shown in Eq. (4)

$$-RT \ln k' = \Delta\mu^o + RT \ln \Phi \quad (4)$$

where Φ is the phase factor characteristic of the MEKC system and independent of solutes. Substitution of $\Delta\mu^o_M$ in Eq. (3) into $\Delta\mu^o$ in Eq. (4) yields Eq. (5), where k' is expressed as a relative value against that of a certain reference compound (k'_0) to eliminate the phase factor, which is also a function of C_n.

$$-RT \ln (k'/k'_0) = (k - k_0)C_n - (\Delta\mu^o_{SDS} - \Delta\mu^o_{SDS,0}) \quad (5)$$

where k_0 and $\Delta\mu^o_{SDS,0}$ denote the corresponding values of the reference compound.

Values of $k-k_0$ for ten substituted benzenes are linear with the concentration of Brij 35 in a SDS–Brij 35 mixed-micellar MEKC system, in which benzaldehyde is used as a reference compound. Table 3 summarizes values of $k-k_0$, which were evaluated from the slopes of the linear plots of $-RT \ln (k'/k'_0)$ against C_n and reflect the relative magnitude of the additional interaction with Brij 35 compared with benzaldehyde. Hydrogen-donating analytes with OH, NH_2 or CH_3CONH substituents have negative values of $k-k_0$, while the other analytes have positive values. The negative values of $k-k_0$ mean that the interaction between the analytes and Brij 35 in the mixed micelle is attractive. The order of the strength of the attractive interaction, as can be judged from Table 3, is fundamentally described in terms of the hydrogen-bonding interactions of the analytes with the polyether chains of Brij 35 in the mixed micelle.

Table 3
Slopes of $-RT \ln (k'/k'_0)$[a] vs. [Brij 35] plots at 293 K (From Ref. [45])

Compound	Slope (kJ mol^{-2})
HO-Ph	−218
4HO-To	−210
NH_2-Ph	−124
4NH_2-To	−93
CH_3CONH-Ph	−52
4CH_3CONH-To	−42
CHO-Ph[a]	0
4CHO-To	24
CH_3COO-Ph	47
4CH_3COO-To	54

[a] CHO-Ph is the reference compound and, thus, k'_0 is the capacity factor of CHO-Ph.

The transfer free energy of a neutral compound in pure SDS systems ($\Delta\mu^o_{SDS}$) will be predominantly governed by the hydrophobic interaction. On the other hand, the transfer free energy of a neutral compound in the SDS–Brij 35 or SDS–Tween 20 mixed-surfactant systems ($\Delta\mu^o_M$) will be governed by the hydrogen-bonding interaction as well as by the hydrophobic interaction and $\Delta\mu^o_M$ is expressed by Eq. (6).

$$\Delta\mu^o_M = \Delta\mu^o_{M,HP} + \Delta\mu^o_{M,HB} \quad (6)$$

where M denotes the mixed micellar system and $\Delta\mu^o_{M,HP}$ and $\Delta\mu^o_{M,HB}$ are the free energies of the hydrophobic interaction and the hydrogen-bonding interaction, respectively.

Brij 35 and Tween 20 in mixed micelles will provide an *attractive* effect and a *repulsive* effect in terms of the hydrogen-bonding and hydrophobic interactions, respectively. The repulsive effect in the hydrophobic interaction results from a decrease in the hydrophobicity of the surface phase of the mixed micelles by the introduction of the polyether chains. Thus, k would be expressed as $k = k_{att} + k_{rep}$, where k_{att} (with a negative sign) and k_{rep} (with a positive sign) are constants representing the attractive and repulsive effects, respectively. Therefore, one can consider that $\Delta\mu^o_{M,HP} = \Delta\mu^o_{SDS} + k_{rep}C_n$ and that $\Delta\mu^o_{M,HB} = k_{att}C_n$.

The energy values listed in Table 3 would be fundamentally considered to reflect the relative strength of the hydrogen-bonding interaction (the k_{att} term), because the analytes used (including the reference compound) have a common hydrophobic benzene moiety and the k_{rep} values of the analytes would be close to each other in this case. However, some contributions of the k_{rep} term are also sug-

gested. A given substituted toluene (X-To) gives a larger $k-k_0$ value than the corresponding benzene with an identical substituent (X–Ph), as judged from Table 3. This would mean that stronger hydrophobic toluene derivatives suffer from greater destabilization in terms of the hydrophobic interaction compared with benzenes. Similar phenomena are observed when the $k-k_0$ values between CH_3COO and CHO substituents and between CH_3CONH and NH_2 substituents are considered.

The separation of hydrophobic cations, such as adrenaline and its precursors, is usually very difficult in SDS-based MEKC systems because the hydrophobic and electrostatic interactions are too strong. In such cases, the addition of non-ionic polyether surfactants is effective for improving the separation, in which the non-ionic polyether surfactants weaken the electrostatic interaction and probably the hydrophobic interaction. The polyether moiety also plays an important role in the improved separation as a hydrogen-bonding acceptor [47].

3. Conclusion

It has been demonstrated that improved separations can be achieved by using some agents with hydrogen-bonding activity as hydrophilic selectors in CZE and MEKC. The hydrogen-bonding interaction is weak in strength in aqueous media, but it can be concluded that the interaction is sufficient to affect the migration pattern of analytes in CE, with the aid of the hydrophobic interaction. The hydrophobic surroundings provided by PEG in PEG-assisted CZE or by micelles in MEKC facilitate the hydrogen-bonding interactions with strong orientation. Hydrogen-bonding interactions, as well as hydrophobic interactions, play an important role in several chiral CE separations using CDs, crown ethers, proteins and surfactants with optically active hydrophilic groups.

Characterization of surfactants based on LSER analysis have also revealed the significance of the hydrogen-bonding interaction. Such hydrogen-bonding effects in PEG-based CZE and mixed-micelle MEKC have been quantitatively expressed. These expressions would be useful for the construction of a separation strategy based on the hydrogen-bonding mode.

In principle, the relative stabilization energy of the hydrogen-bonding interaction is increased in non-aqueous media compared to aqueous media. Therefore, the development of such a hydrogen-bonding mode in a non-aqueous CE separation system is interesting and may become important in the future. Inclusion phenomena of cations by polyethers is also reported as an electrostatic coordination interaction in non-aqueous media [54]. On the other hand, dipole–dipole and dipole-induced dipole interactions, as weak electrostatic interactions that are not able to function sufficiently in aqueous media, would also be candidates for the new separation modes in non-aqueous CE [55,56]. All of these interactions work cooperatively in CE and the function and ability of selectors will depend on the medium. This suggests that a variety of separation modes will be developed in future by the combination of selectors and medium.

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