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Effects of organic modifiers on retention mechanism and selectivity in micellar electrokinetic capillary chromatography studied by linear solvation energy relationships

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Abstract

The effects of six organic modifiers (urea, methanol, dioxane, tetrahydrofuran, acetonitrile and 2-propanol) on the retention mechanism and separation selectivity of the bulk buffer in micellar electrokinetic capillary chromatography (MECC) with sodium dodecyl sulfate (SDS) micelles as pseudo-stationary phase have been investigated through linear solvation energy relationships (LSERs). It is found that the retention value in MECC systems with or without organic modifier is primarily dependent on the solvophobic interaction and the hydrogen bonding interaction with the solute as proton acceptor, while the dipolar interaction and the hydrogen bonding interaction with the solute as proton donor play minor roles. The effects of the organic modifiers on the solvophobic, dipolar and hydrogen bonding interactions are evaluated in terms of the relationship between regression coefficient of the LSER equations and the modifier concentration. The variations of the solvophobic interaction and the dipolar interaction with change of the modifier concentration can be approximately explained using the solubility parameter and the dipolarity/polarizability parameter of the organic modifier, respectively. However, the relationships between the hydrogen bond acidity and basicity of the bulk buffer and the organic modifiers are rather complicated. Those results may be caused from the displacement of organic modifiers to the water adsorbed on the micellar surface as well as changes in the acidity and basicity of the bulk buffer with the addition of organic modifiers. In addition, it is found that the phase ratio is influenced significantly by the use of organic modifier.

Keywords: Organic modifiers; Selectivity; Retention mechanisms; Micellar electrokinetic chromatography; Linear solvation energy relationships; Buffer composition

1. Introduction

Micellar electrokinetic capillary chromatography (MECC) has been developed into a highly efficient method for the separation of electrically neutral compounds. Uncharged compounds can be separated based on the differences in their partitioning between the micellar phase (the stationary phase) and the bulk buffer solution (the mobile phase) in MECC. For a common analysis task, sodium dodecyl sulfate (SDS) is the most versatile surfactant while organic modifiers are widely used as buffer components to

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improve the separation. Thus the type and concentration of organic modifiers are usually the main factors for the optimization of separation conditions. The roles of organic modifiers in MECC have been investigated extensively [1–13]. It has been found that organic modifiers can expand the migration-time window, decrease solute retention and alter separation selectivity, achieve gradient elution, and even improve peak shape. The retention mechanism and the chemical selectivity of various surfactants in MECC have been studied [14–18], however, the effects of organic modifiers on the retention mechanism and the selectivity of the bulk buffer are still scarcely known. The main focus of this paper is to investigate the effects of six organic modifiers, i.e., urea, methanol (MeOH), dioxane (DIO), acetonitrile (ACN), tetrahydrofuran (THF) and 2-propanol (2-PrOH), on the retention mechanism and the chemical selectivity of the mobile phase in MECC through linear solvation energy relationships (LSERs).

2. Experimental

2.1. Instrumentation and separation conditions

All experiments were carried out on a Beckman P/ACE system 5010 (Fullerton, CA, USA). System Gold software running on a personal computer was used to collect data and control the instrument. The temperature was kept constant at 25±0.1°C. The wavelength of the UV detector was set at 214 nm. Injections were made in the pressure mode (3.45 kPa) for 2 s. All experiments were carried out at a constant voltage of 25 kV by placing the anode at the inlet side and the cathode at the outlet side of the capillary. Fused-silica capillaries of 47 cm (40 cm to detector)×50 μm I.D. (Yongnian Optical Fiber Plant, Hebei, China) were used.

2.2. Samples and solutions

Thirty-one substituted benzenes with different functional groups and different solvatochromic parameters were selected as probe solutes as shown in Table 1. The reagents ethylbenzene, biphenyl, pyrene, benzaldehyde, acetophenone, propiophenone, anisole, ethoxylbenzene, benzyl alcohol, 3-phenylpropanol, *p*-nitrophenol, *p*-nitrotoluene and *p*-dichlorobenzene were of chemical-reagent grade, while all of the others used were of analytical-reagent grade. The concentrations of the probe solutes were about 0.1–1 mg/ml. SDS and all the six organic modifiers were of analytical-reagent grade. The running buffers were 50 mmol/l SDS–20 mmol/l sodium tetraborate solutions containing different concentrations of organic modifiers, prepared by the addition of organic modifiers of required concentrations prior to adjusting the pH to 9.00 with 0.1 mol/l HCl and 0.1 mol/l NaOH solutions. The concentration series of organic modifiers were: urea, 0, 1.000, 2.000, 3.000, 4.000 and 5.000 mol/l; MeOH, 0, 1.416, 2.596 and 3.893 mol/l; DIO, 0, 0.583, 1.167 and 1.750 mol/l; ACN, 0, 0.489, 0.978,

Table 1
The probe solutes and their structural parameters[a]

Solute	V	π^*	β	α
Benzene	0.491	0.59	0.10	0.00
Toluene	0.592	0.55	0.11	0.00
Ethylbenzene	0.668	0.53	0.12	0.00
Propylbenzene	0.769	0.51	0.12	0.00
Butylbenzene	0.867	0.49	0.12	0.00
Biphenyl	0.920	1.18	0.20	0.00
Pyrene	1.156	0.90	0.25	0.00
Pyridine	0.470	0.87	0.44	0.00
Benzaldehyde	0.606	0.92	0.44	0.00
Acetophenone	0.690	0.90	0.49	0.04
Propiophenone	0.788	0.88	0.49	0.00
Butyrophenone	0.886	0.86	0.49	0.00
Benzonitrile	0.590	0.90	0.37	0.00
Anisole	0.639	0.73	0.32	0.00
Ethoxylbenzene	0.727	0.69	0.30	0.00
Naphthalene	0.753	0.70	0.15	0.00
Phenol	0.536	0.72	0.33	0.61
p-Methylphenol	0.634	0.68	0.34	0.58
Methyl benzoate	0.736	0.75	0.39	0.00
Ethyl benzoate	0.834	0.74	0.41	0.00
Benzyl alcohol	0.634	0.99	0.52	0.39
2-Phenylethanol	0.732	0.97	0.55	0.33
3-Phenylpropanol	0.830	0.95	0.55	0.33
Nitrobenzene	0.631	1.01	0.30	0.00
p-Nitrophenol	0.676	1.15	0.32	0.82
p-Nitrotoluene	0.729	0.97	0.31	0.00
Aniline	0.562	0.73	0.50	0.26
p-Nitroaniline	0.702	1.25	0.48	0.42
Chlorobenzene	0.581	0.71	0.07	0.00
Bromobenzene	0.624	0.79	0.06	0.00
p-Dichlorobenzene	0.671	0.70	0.03	0.00

[a] The structure parameters are taken from Ref. [19].

1.466 and 1.760 mol/l; THF, 0, 0.308, 0.616, 0.924 and 1.231 mol/l; 2-PrOH, 0, 0.327, 0.654, 1.308 mol/l. Further higher concentrations of organic modifier cannot be employed, due to the fact that the SDS micelles migrate to the anode at a higher concentration of organic modifier in the buffer system studied. In order to conveniently compare the effects of urea with those of the other modifiers at identical concentrations, the unit of molar concentration was used, unless otherwise stated. All running buffers were filtered through 0.45-μm membrane filters. All solutions were prepared with ultra-pure water, produced by a Milli-Q water system (Millipore, Bedford, MA, USA).

2.3. Methods

New capillaries were activated by rinsing with 1 mol/l NaOH, 0.1 mol/l NaOH and ultra-pure water for 2 h, 30 min and 30 min, respectively. At the beginning of experiments each day, the capillary was re-activated with each of the solutions for 20 min. Pre-rinse was done before injection with 1 mol/l NaOH for 5 min, ultra-pure water for 3 min and then the running buffer for 5 min. Formamide was selected as the dead time tracer. Usually, highly hydrophobic compounds that are retained completely in micelles such as Sudan III and dodecanophenone are used as tracer for migration time of micelle (t_{mc}). However, when a high concentration of organic modifier is used, the t_{mc} tracer will be eluted to some extent by the bulk solution. Therefore, in this paper the iterative procedure [20] was employed to calculate the migration time of SDS micelles with a homologous series of alkylbenzenes on a personal computer. The iterative procedure was set to stop when a converged value was obtained with a difference less than 0.001 min between consecutive values of t_{mc}.

2.4. Calculation of capacity factor of solute

The capacity factor (k') for uncharged solutes was calculated according to the following formula [21–23]:

$$k' = \frac{t_r - t_0}{(1 - t_r/t_{mc})t_0} \quad (1)$$

where t_0, t_r and t_{mc} are the migration times of an unretained substance (the dead time), the solute and the micelle, respectively.

3. Results and discussion

3.1. LSER methodology

LSERs, developed by Kamlet and co-workers [24,25], have been applied extensively to study the retention mechanisms in gas chromatography (GC) [26–31], reversed-phase liquid chromatography (RPLC) [32–45], normal-phase liquid chromatography (NPLC) [46,47], micellar liquid chromatography [48] and MECC [14–18,49]. When the LSER approach is applied to a MECC system, the logarithmic capacity factor, log k', can be separated into several molecular interaction terms as shown as follows:

$$\log k' = \log k'_0 + M(\delta_b^2 - \delta_m^2)V_s + S(\pi^*_m - \pi^*_b)\pi^*_s + B(\alpha_m - \alpha_b)\beta_s + A(\beta_m - \beta_b)\alpha_s \quad (2)$$

The subscripts b, m and s denote the properties of bulk buffer, micellar phase and solute, respectively. Log k'_0 is the intercept while M, S, B and A are the fitting parameters of the multiple linear regression. δ^2 is the Hildebrand solubility parameter which measures the cohesiveness of the chromatographic phases (both the bulk buffer phase and the micellar phase). V_s is the molecular volume of solute. π^*, α and β represent the dipolarity/polarizability, hydrogen bond acidity and hydrogen bond basicity of the chromatographic phases and the solute. The $M(\delta_b^2 - \delta_m^2)V_s$ term, called the cavity term, is a measure of the endoergic (unfavorable) process of separating the solvent or the micellar phase molecules to provide a suitably sized enclosure for the solute. It reflects the cohesiveness difference between the two phases. The dipolarity/polarizability term, $S(\pi^*_m - \pi^*_b)\pi^*_s$, measures the exoergic (favorable) effects of the dipole–dipole and dipole–induced dipole interactions between the solutes and the bulk phases. The exoergic hydrogen bonding term, $B(\alpha_m - \alpha_b)\beta_s$, measures the effect of complexation between hydrogen bond acceptor (HBA) solutes and hydrogen bond donor

(HBD) bulk phases. The $A(\beta_m - \beta_b)\alpha_s$ term, another hydrogen bonding term, measures the exoergic effect of complexation between HBD solutes and HBA bulk phases. For the case of a fixed pair of bulk buffer and micellar phases, Eq. (2) can be reduced to Eq. (3) as follows

$$\log k' = \log k'_0 + mV_s + s\pi^*_s + b\beta_s + a\alpha_s \quad (3)$$

where *m*, *s*, *b* and *a* are fitting coefficients characteristic of the pair of chromatographic phases, called system coefficients. In comparing Eq. (3) with Eq. (2), it can be known that each coefficient in Eq. (2) reflects the difference in a specific bulk property between the bulk solution and the micellar phase. In other words, the coefficients *m*, *s*, *b* and *a* provide quantitative information about solute–micelle, solute–buffer interactions and selectivity of the bulk buffer in MECC. In addition, the intercept log k'_0 includes information about the phase ratio [50]. From the magnitude of the system coefficients and the intercept, the interactions governing the retention and the effects of organic modifiers on the phase ratio can be known.

3.2. Retention mechanism

The solvatochromic parameters of SDS, water and the organic modifiers used in this work are listed in Table 2. Prediction of the system coefficients in Eq. (3) for different surfactant–buffer–modifier systems from the solvatochromic parameters is very difficult because the properties of the stationary and mobile phases in chromatographic systems are more complicated than those in homogeneous and pure state. For example, Tan et al. [44] found that sorption of water and organic modifier onto the stationary phase in reversed-phase high-performance liquid chromatography (RP-HPLC) strongly influences properties of the stationary phase. Therefore, the system coefficients should be measured by multiparameter simultaneous least-square regressions of the log k' values of a set of solutes against their solvatochromic parameters.

LSER analyses for all the organic modifier concentrations are carried out and the results are listed in Table 3. It can be seen that the log k' values are correlated well with the solute solvatochromic parameters, most of the correlation coefficients are higher than 0.99. However, the robustness of the LSER models is a little worse than that for HPLC; the standard deviations of the system coefficients and the average residuals for MECC systems are rather larger than those for HPLC systems reported in the literature even at the same correlation coefficient level. Such a problem can be also found in the LSER equations for MECC reported by other authors [14–18], the reason for which is not clear at present. As can be seen in the LSER equations shown in Table 3, the coefficients *m* and *b* are very large while the coefficients *s* and *a* are quite small, which means that the retention of solute is primarily dependent on the solvophobic interaction and the hydrogen bonding interaction with the solute as proton acceptor, while the dipolar interaction and the hydrogen bonding interaction with the solute as proton donor play minor roles. This is consistent with the results obtained by Khaledi and co-workers [14,15,18], Zou et al. [16] and Muijelaar et al. [17] for MECC systems without addition of any organic modifier in the running buffer. Therefore, the separation selectivity of bulk buffer in MECC system with SDS micelles as pseudostationary phase mainly depends on the differences in the solvophobic selectivity and

Table 2
The solubility parameters and the solvatochromic parameters for SDS anion, water and organic modifiers[a]

Compound	δ	V	π^*	β	α
$CH_3(CH_2)_{10}CH_2SO_4^-$[b]	–	–	1.00	0.88	0.00
Water	23.4	–	1.09	0.18	1.17
Urea[c]	–	0.265	0.90	0.74	0.76
MeOH	14.3	0.205	0.60	0.62	0.93
DIO	10.1[d]	0.410	0.55	0.41	0.00
THF	9.33	0.455	0.58	0.55	0.00
ACN	11.8	0.271	0.75	0.31	0.19
2-PrOH	11.5	0.402	0.48	0.68	0.76

[a] The solubility parameters are taken from Ref. [50] while the solvatochromic parameters are taken from Ref. [51].
[b] The solvatochromic parameters were predicted according to Ref. [52].
[c] The solvatochromic parameters were taken from Ref. [52].
[d] The solubility parameter was taken from Ref. [53].

Table 3
Coefficients of LSER equations for all conditions of organic modifier[a]

Organic modifier	log k'_0	m	s	b	a	n	R	sd
No	−1.549±0.106	4.058±0.130	−0.141±0.112	−2.114±0.128	−0.109±0.086	31	0.9914	0.095
1.000 mol/l urea	−1.504±0.098	3.870±0.120	−0.161±0.103	−2.033±0.118	−0.113±0.080	31	0.9920	0.087
2.000 mol/l urea	−1.482±0.093	3.740±0.114	−0.188±0.098	−1.977±0.112	−0.096±0.075	31	0.9923	0.083
3.000 mol/l urea	−1.475±0.090	3.638±0.110	−0.207±0.095	−1.942±0.109	−0.073±0.073	31	0.9924	0.080
4.000 mol/l urea	−1.478±0.089	3.575±0.109	−0.233±0.094	−1.911±0.108	−0.057±0.073	31	0.9923	0.080
5.000 mol/l urea	−1.487±0.089	3.509±0.108	−0.251±0.094	−1.883±0.107	−0.035±0.072	31	0.9921	0.079
1.416 mol/l MeOH	−1.563±0.113	3.919±0.138	−0.088±0.121	−2.258±0.137	−0.036±0.093	30	0.9903	0.100
2.596 mol/l MeOH	−1.568±0.101	3.875±0.124	−0.082±0.107	−2.354±0.122	−0.018±0.082	31	0.9919	0.090
3.893 mol/l MeOH	−1.406±0.096	3.374±0.117	−0.059±0.101	−2.240±0.116	−0.026±0.078	31	0.9911	0.085
0.583 mol/l DIO	−1.407±0.099	3.572±0.121	−0.106±0.104	−2.232±0.120	0.009±0.080	31	0.9911	0.088
1.167 mol/l DIO	−1.340±0.086	3.393±0.106	−0.096±0.091	−2.380±0.104	0.045±0.070	31	0.9930	0.077
1.750 mol/l DIO	−1.368±0.104	3.129±0.148	−0.041±0.100	−2.295±0.110	0.044±0.073	29	0.9873	0.080
0.308 mol/l THF	−1.389±0.091	3.842±0.111	−0.113±0.096	−2.521±0.110	0.028±0.074	31	0.9936	0.081
0.616 mol/l THF	−1.311±0.093	3.734±0.114	−0.073±0.098	−2.711±0.113	0.086±0.076	31	0.9934	0.083
0.924 mol/l THF	−1.203±0.096	3.548±0.117	−0.064±0.101	−2.768±0.116	0.128±0.078	31	0.9926	0.085
1.231 mol/l THF	−1.111±0.102	3.330±0.125	−0.021±0.108	−2.762±0.123	0.160±0.083	31	0.9909	0.091
0.489 mol/l ACN	−1.471±0.098	3.921±0.120	−0.131±0.103	−2.218±0.118	−0.089±0.079	31	0.9925	0.087
0.978 mol/l ACN	−1.449±0.093	3.838±0.114	−0.122±0.098	−2.308±0.113	−0.054±0.076	31	0.9931	0.083
1.466 mol/l ACN	−1.401±0.088	3.692±0.107	−0.113±0.093	−2.332±0.106	−0.040±0.071	31	0.9935	0.078
1.760 mol/l ACN	−1.380±0.090	3.502±0.119	−0.113±0.083	−2.223±0.096	−0.036±0.064	30	0.9924	0.070
0.327 mol/l 2-PrOH	−1.402±0.101	3.740±0.123	−0.084±0.106	−2.238±0.122	−0.271±0.082	31	0.9919	0.090
0.654 mol/l 2-PrOH	−1.309±0.100	3.631±0.122	−0.134±0.105	−2.341±0.121	−0.234±0.081	31	0.9920	0.089
1.308 mol/l 2-PrOH	−1.189±0.108	3.402±0.133	−0.113±0.114	−2.530±0.131	−0.191±0.088	31	0.9903	0.097

[a] n Is the number of test solutes; R is the correlation coefficient of linear regression; sd is the average residual.

HBD acidic selectivity between the micellar phase and the bulk buffer.

3.3. Effects of organic modifiers on molecular interactions

The coefficients in Eq. (3) change with the concentration of organic modifier in buffer, which means that the separation selectivity of MECC systems can be adjusted by changing the modifier concentration. Because the standard deviations for each system coefficient under different conditions are nearly at the same level, the effects of organic modifiers on the intermolecular interactions and the selectivity can be examined in terms of the dependence of the system coefficients on the type and concentration of organic modifiers.

3.3.1. Effects of organic modifiers on hydrophobic interaction

The cavity term essentially reflects the solvophobic interaction (solvophobic interaction is sometimes called hydrophobic interaction, but the common term is solvophobic interaction [53]). For a given solute, the solvophobic interaction is mainly dependent on the difference between the cohesiveness of the micelle and that of the bulk buffer. In general, the cohesiveness of organic compounds is mainly dependent on the alkyl chain in the molecule; and the longer the alkyl chain, the less the cohesiveness. It can be expected that the cohesiveness of SDS

is less than the corresponding property of water. Therefore, the solvophobic interaction tends to keep the solutes in the micellar phase. As shown in Table 3, the coefficient *m* observed for all the modifier–buffer systems has a large positive value.

Considering the organic modifiers as solutes, the amount of the organic modifiers dissolved in the micellar phase can be estimated from the LSER equations obtained. Substituting the solvatochromic parameters of organic modifiers into the LSER equations listed in Table 3 gives the capacity factors of 0.006, 0.007, 0.089, 0.075, 0.061 and 0.018 for the organic modifiers of urea, MeOH, DIO, THF, ACN and 2-PrOH at concentrations of 5.000, 3.893, 1.750, 1.760, 1.231 and 1.308 mol/l, respectively. The very small capacity factors of the organic modifiers indicate that the amount of organic modifier dissolved in the micellar phase is very limited. These results are consistent with the conclusions that urea, MeOH, ACN and DIO are non-penetrating additives for SDS micelles [54,55]. Therefore, one can conclude that organic modifiers have little influence on the cohesiveness of the micelle. Thus variation of the coefficient *m* is mainly dependent on the change of the cohesiveness of the bulk buffer. It can be seen from Table 2 that the solubility parameter of the organic modifier is much less than that of water. Therefore, addition of organic modifier will lead to a decrease in the coefficient *m*, that is, the solvophobic interaction is decreased. If the concentrations employed are the same, an organic modifier having a lower solubility parameter should have a greater ability to decrease the solvophobic interaction.

The *m* values are plotted against the concentration of organic modifiers as shown in Fig. 1. For the five organic modifiers of urea, DIO, THF, ACN, 2-PrOH, the plots of the *m* values versus the concentrations of organic modifiers are approximately linear, while the plot of the *m* versus MeOH concentration is curvilinear. But in all the cases, the *m* values decrease with increasing concentrations of organic modifiers. For the former five organic modifiers, the slopes of the linear relationships observed can be used to characterize the abilities of the organic modifiers to decrease the solvophobic interaction. Fig. 1 shows that the abilities follow the order of urea<ACN<2-PrOH<DIO<THF, which is consistent with the above prediction.

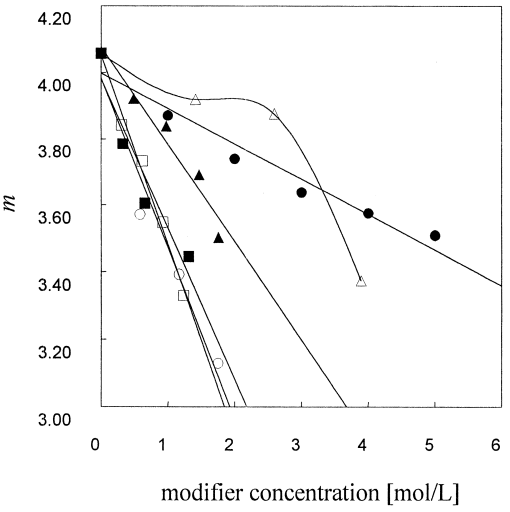

Fig. 1. Dependence of the coefficient *m* on the molar concentration of organic modifier. Organic modifiers: ●, urea; △, MeOH; ○, DIO; □, THF; ▲, ACN; ■, 2-PrOH.

3.3.2. Effects of organic modifiers on dipolar interaction

The dipolar interaction of a given solute is dependent on the dipolarity/dipolarizability difference between the bulk buffer and the micellar phase. It can be seen from Table 2 that the π^* value of the SDS anion is slightly less than that of the solvent water. Therefore, the coefficient *s* for a MECC system without organic modifier should be a small negative value. This view is consistent with the observed *s* value. Cheong and Carr [56] measured the dipolarity/dipolarizability of binary organic–aqueous mobile phases used in RP-HPLC and the results showed that the π^* value of organic–aqueous mixture roughly linearly decreases with increasing organic modifier concentration, but remains the same for the 2-PrOH–water mixture within the range from 0 to 20% (v/v) of 2-PrOH. It is assumed that the π^* value of organic modifier (except 2-PrOH)–aqueous mixture can be estimated approximately by the following equation:

$$\pi^*_{mix} = \pi^*_w \phi_w + \pi^*_o \phi_o \quad (4)$$

where π^*_{mix}, π^*_w and π^*_o are the dipolarity/dipolarizability of the mixture, water and organic solvent, respectively. ϕ_w and ϕ_o are the volume fraction of water and organic solvent, respectively. The π^* value of the bulk buffers containing MeOH, DIO, ACN and THF is calculated according to Eq. (4), and all the π^* values of these bulk buffers are found to be slightly lower than that of water but a little higher than that of the SDS anion ($1.009 < \pi^*_{mix} < 1.078$). Actually, the micellar surface may adsorb some amount of organic modifier, leading to a π^* value of the micellar phase being lower than that without organic modifier in the buffer. Therefore, the coefficient *s* for these systems should be also negative. The prediction is confirmed by the *s* values listed in Table 3. It can be expected from Eq. (4) that for the systems containing MeOH, DIO, ACN and THF the coefficient *s* should linearly increase with modifier concentration. In Fig. 2 such an expectation is verified with the linear plots, of which the correlation coefficients for the organic modifiers are 0.949, 0.966, 0.987 and 0.986, respectively. For the systems with 2-PrOH as modifier, the data point of 0.327 mol/l seems to be abnormal. If it is omitted, a linear relationship between the coefficient *s* and 2-PrOH concentration will be observed.

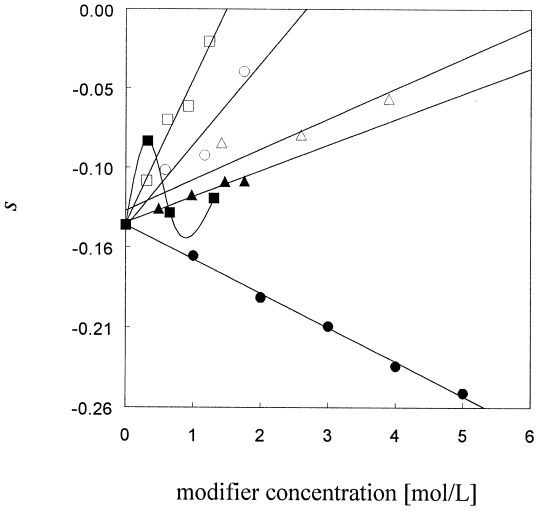

Fig. 2. Dependence of the coefficient *s* on the molar concentration of organic modifier. Organic modifiers: ●, urea; △, MeOH; ○ DIO; □, THF; ▲, ACN; ■, 2-PrOH.

Fig. 2 shows that the addition of these five kinds of organic modifiers including 2-PrOH, DIO, THF, MeOH and ACN leads to an increase in the coefficient *s*, that is, the dipolar interaction between the solute and the bulk buffer decreases. As a contrast, it is found that the existence of urea causes an increase in the dielectric constant of aqueous solution [57], which means that the dipolarity/dipolarizability of bulk buffer will increase with addition of urea. Thus the coefficient *s* for the bulk buffer containing urea should be also a negative value but less than that for the system without organic modifier, which is also supported by the values of coefficient *s* shown in Table 3. When the coefficient *s* is plotted against urea concentration, a linear relationship is observed as shown in Fig. 2, giving a correlation coefficient of 0.999. Therefore, when urea is used as the modifier, the dipolar interaction between the solute and the bulk buffer linearly increases as urea concentration increases. It can be observed that the negative value of the slope in Fig. 2 indicates the abilities of the organic modifiers to increase the dipolar selectivity of the bulk buffer follow the order: THF < DIO < MeOH < ACN < urea.

3.3.3. Effects of organic modifiers on HBD phases–HBA solute hydrogen bonding interaction

The coefficient *b* is dependent on the relative strength of HBD acidity of the micelle to the bulk buffer. Although the SDS anion has no HBD acidity, the SDS micelle has HBD acidity because water molecules may be adsorbed onto the micellar surface through dipolar interaction and hydrogen bonding interaction. However, the total HBD acidity of the micellar phase should be much lower than that of the bulk buffer. Therefore, coefficient *b* should be a large negative value. The observed *b* value is consistent with the expectation. The *b* values are plotted against the concentrations of organic modifiers as shown in Fig. 3. Obviously, the coefficient *b* is decreased by the addition of MeOH, DIO, ACN, THF and 2-PrOH; especially in the case of THF, where the biggest drop is observed. However, the *b* value increases as urea concentration increases. There are two ways to change the *b* value by addition of organic modifier into aqueous buffer. One is that the adsorption of organic modifier on

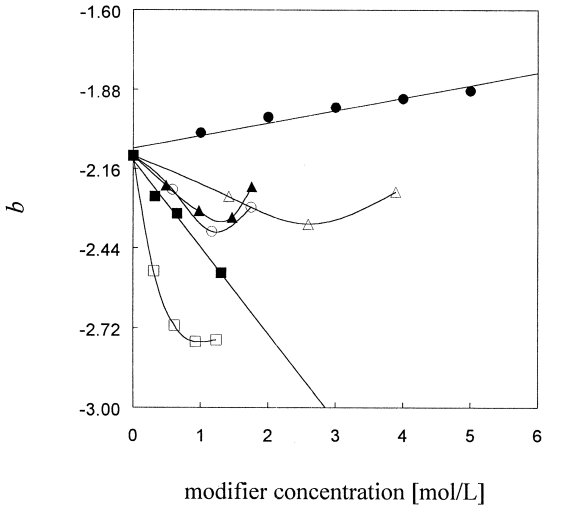

Fig. 3. Dependence of the coefficient *b* on the molar concentration of organic modifier. Organic modifiers: ●, urea; △, MeOH; ○, DIO; □, THF; ▲, ACN; ■, 2-PrOH.

micellar surface will displace the adsorbed water, which leads to lower acidity of micellar surface and further to decrease the *b* value due to much lower acidity of adsorbed organic modifier than that of adsorbed water. Secondly, the addition of organic modifiers into the aqueous buffer will break the water complexes associated through hydrogen bonding, and the organic modifier will interact with water, which leads to a change in the buffer acidity [58]. Although the knowledge about the internal delicate structure of organic–aqueous buffers, even of water is still unclear, it was observed that the acidity of binary mobile phase decreases with increasing concentration of organic modifiers in RP-HPLC [56], which leads to higher *b* values. From the results shown in Fig. 3, it is known that the negative contribution to the coefficient *b* from the displacement effect of organic modifier to the adsorbed water on micellar surface is larger than the positive contribution from the change of buffer acidity by addition of MeOH, DIO, ACN, THF and 2-PrOH into the buffer, but the positive contribution to the coefficient *b* is higher than the negative contribution if urea is added into the buffer. This may be caused by the fact that urea is much more hydrophilic than the other organic modifiers used, which leads to less change of micellar acidity but to a considerable decrease in the acidity of the bulk buffer. Although the relationship between the HBD acidity and the buffer composition is complicated, it was found that the THF–buffer is the strongest while the urea–buffer is the weakest in HBD acidic selectivity among the organic–aqueous buffers studied.

3.3.4. Effects of organic modifiers on HBA phases–HBD solute hydrogen bonding interaction

The coefficient *a* is dependent on the difference of HBA basicity between the bulk buffer and the micellar phase. It can be seen from Table 2 that the β value of SDS micelle is much larger than that of water and the six organic modifiers used. So the coefficient *a* should be positive under aqueous buffer. However, the coefficient *a* under aqueous buffer takes a small negative value, which may be caused by two reasons: (1) the adsorbed water on the micellar surface leads to lower basicity of the micellar phase due to the much lower basicity of water than that of SDS; and (2) the free SDS molecules in the buffer lead to higher basicity of the bulk buffer. The effects of organic modifier concentration on the *a* value are shown in Fig. 4. It can be seen that the addition of urea, MeOH, ACN, DIO and THF into the buffer makes a positive contribution to the coefficient *a*, which means that the

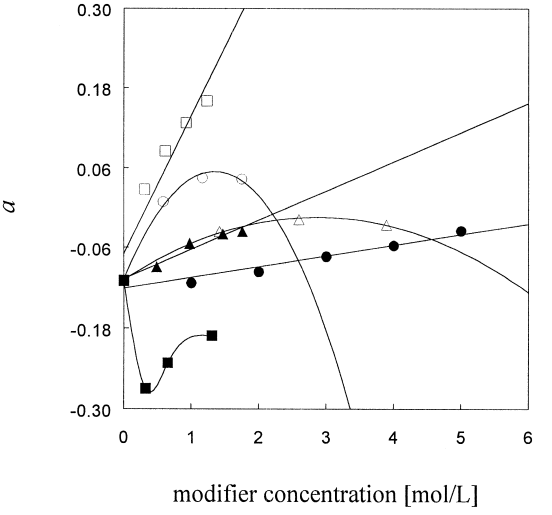

Fig. 4. Dependence of the coefficient *a* on the molar concentration of organic modifier. Organic modifiers: ●, urea; △, MeOH; ○, DIO; □, THF; ▲, ACN; ■, 2-PrOH.

addition of those modifiers increases the basicity of the micellar phase or decreases the basicity of the bulk buffer. As stated above, the adsorption of organic modifier on the micellar surface will displace the adsorbed water, which will lead to an increase in the basicity of micellar surface due to the higher basicity of organic modifiers than that of water. On the other hand, addition of organic modifiers into the aqueous buffer will also lead to change of the buffer basicity, which is unclear at the present time. However, the addition of 2-PrOH into the buffer leads to lower *a* values, which is difficult to explain. It may probably be caused by the uncertainty of the small *a* values during regression analysis. Fig. 4 evidently shows that THF is the most effective organic modifier to adjust the HBA basicity of bulk buffer among the organic modifiers studied.

3.4. Effects of organic modifiers on phase ratio

According to the LSER method, the intercept log k_0' is related to the chromatographic phase ratio [50]. Thus change in log k_0' shows the variation of MECC phase ratio. The phase ratio (Φ) in MECC is expressed as:

$$\Phi = V_{mc}/V_b \quad (5)$$

where V_{mc} and V_b are the total volume of the micellar phase and the buffer phase, respectively. The total micellar phase volume can be calculated approximately according to the following formula:

$$V_{mc} = (C_{surf} - CMC)V_c N_0 v_{mc}/N \quad (6)$$

where C_{surf} and CMC are total concentration and critical micelle concentration of the surfactant, respectively, V_c is the inner volume of the capillary, N_0 is Avogadro constant, v_{mc} is the average volume of the micelle, N is the aggregation number of the micelle. It can be seen from Eqs. (5) and (6) that the effect of organic modifier on the phase ratio mainly results from the changes of CMC, v_{mc} and N. It was found that the CMC of SDS increased with the concentration of MeOH, DIO and urea in water [55,59,60], e.g., the CMC of SDS in 6 mol/l urea at 25°C increased by 1.67-times relative to that in water [60]. Thus the increase of CMC resulted from the addition of organic modifier will lead to a decrease in the phase ratio. Secondly, it has been found that the aggregation number of micelles decreases in the presence of organic modifiers [61–63], and the micelle volume should be proportional to the aggregation number of micelles. Therefore, the addition of organic modifiers has a positive contribution to the phase ratio directly from the decrease in the aggregation number, but a negative contribution from the decrease of the micelle volume induced by the decrease of the aggregation number. Finally, the cohesiveness of the buffer is reduced by the addition of organic modifiers because they have lower cohesiveness, thus the compressive force on the micelle is thereby decreased, which will lead to increase of the micelle volume. In this respect, the addition of organic modifiers has positive contribution to the phase ratio through its effect on the micelle volume. It can be concluded based on the above discussion that the effect of organic modifiers on the phase ratio should be relatively complicated. The dependence of the intercept log k_0' on the concentration of organic modifier is shown in Fig. 5. The plots of log k_0' against the modifier concentration for ACN, THF and 2-PrOH are linear, whereas the corresponding plots for urea, MeOH and DIO are curvilinear. The above results indicated that the phase ratio of MECC system strongly depends on the type and concen-

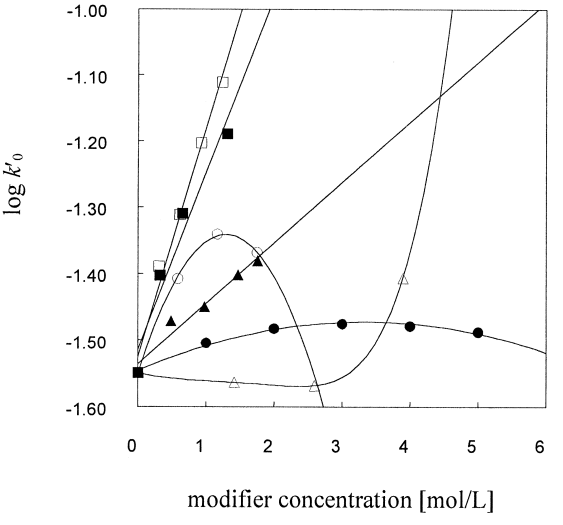

Fig. 5. Dependence of the intercept log k' on the molar concentration of organic modifier. Organic modifiers: ●, urea; △, MeOH; ○, DIO; □, THF; ▲, ACN; ■, 2-PrOH.

tration of the organic modifier used. Thus it is clear that organic modifiers influence the solute retention by changing not only the separation selectivity but also the phase ratio.

4. Conclusion

The LSER approach has been successfully used to investigate the effects of organic modifiers on the retention mechanism and selectivity of the bulk buffer in MECC. In MECC systems with or without organic modifiers, the retention of solute is determined dominantly by the molecular volume and HBA basicity of solute, that is, the separation selectivity is determined predominantly by the solvophobic selectivity and HBD acidic selectivity of micellar phase and bulk buffer. The changes in solvophobic and dipolar interactions are influenced mainly by the cohesiveness and dipolarity/dipolarizability of organic modifier, respectively. However, changes in the hydrogen bonding interactions result from not only the hydrogen bond properties of organic modifier but also probably the interactions between the solvent water, the organic modifier and the micelle. As for the selectivity, we refer to the selectivity of the entire bulk buffer instead of that of the organic modifier used, because the selectivity of bulk buffer, especially the acidic and basic selectivity, is rather different from that of pure organic modifier. It is found that THF has the strongest ability to adjust the solvophobic, acidic and basic selectivity, while urea has the strongest ability to adjust the dipolar selectivity of bulk buffer. Therefore, the greatest variation in selectivity can be expected to occur in a ternary organic–aqueous buffer containing THF and urea simultaneously. On the other hand, the use of organic modifier significantly changes the phase ratio of MECC, which also contributes to the change of solute retention.

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