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Characterization of Surfactant Selectivity in Micellar Electrokinetic Chromatography

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The solvation parameter model is used to delineate the contribution of individual intermolecular interactions to the retention properties of seven common surfactants used in micellar electrokinetic chromatography. Buffer composition and concentration, pH, temperature, and voltage had only a small influence on selectivity for sodium cholate micelles in the normal experimental range for separations. Surfactant concentration was found to affect retention by changing the phase ratio without significantly changing selectivity while the addition of organic solvent in low concentration (up to 10% v/v) has an influence on the solvophobic properties of the buffer (lowers cohesion and reduces its capacity as a hydrogen-bond acid) in a manner which seems to depend very little on solvent identity. The bile salt surfactants, sodium cholate, sodium deoxycholate, sodium taurocholate, and sodium deoxytaurocholate, are quite similar as a group, exhibiting modest differences in their cohesion and hydrogen-bond acidity. Sodium dodecyl sulfate has complementary properties to the bile salt surfactants; it is less cohesive and a weaker hydrogen-bond base and a stronger hydrogen-bond acid. The sodium salt of *N*-dodeconyl-*N*-methyltaurine is a stronger hydrogen-bond base than the support buffer and weak hydrogen-bond acid; again properties that set it apart from the bile salts and sodium dodecyl sulfate for the separation of hydrogen-bonding solutes. Hexamethyltrimethylammonium bromide is the least cohesive and the strongest hydrogen-bond base of the surfactants studied but its relatively small migration window may make it less useful in practice for complex separations. Lithium perfluorooctanesulfonate has rather unique selectivity (high cohesion, significant capacity for dipole-type interactions and strong hydrogen-bond acidity) that would make it a good choice with the other surfactants for general selectivity optimization in methods development.

Keywords: *Micellar electrokinetic chromatography*; *surfactant selectivity*; *solvation parameter model*; *experimental parameters that influence selectivity*; *surfactant selection*

Micellar electrokinetic chromatography (MEKC) is a member of a family of recently introduced separation techniques using electrophoretic or electroosmotic principles as the basis of the differential migration mechanism resulting in separation.[1–4] The distinguishing feature of MEKC is the addition of a surfactant above its critical micelle concentration to the separation buffer such that in an electric field the migration velocity of the micelles and the bulk electrolyte in the direction of general flow are different. Neutral solutes can then be separated if they have different distribution constants between the bulk electrolyte and the micelle phase and will be eluted within the migration window established by the difference in the velocity between the micelle phase and the bulk electrolyte. In the same system ionized solutes can be separated by a combination of differences in their distribution between phases and their effective electrophoretic mobility. The high kinetic efficiency and the possibility of separating both neutral and ionized solutes in the same system are a considerable attraction of MEKC and this combined with the flexibility of adjusting selectivity that can be achieved by adding complexing agents (*e.g.*, cyclodextrins, urea, *etc.*), different surfactants or organic solvents to the separation buffer, has resulted in an extensive number of practical applications. The commonality of the instrumentation for MEKC and capillary electrophoresis has removed any hurdles to the use of micellar-based separation systems in laboratories equipped for capillary electrophoresis.

Although many acceptable separations have been published by MEKC the general approach to methods development is largely based on trial and error experiments assisted by some useful formal general observations.[2,3,5–8] Resolution depends mainly on the choice of the surfactant system, operation under conditions resulting in an acceptable migration window, and maintenance of experimental conditions that provide high kinetic efficiency. These parameters in turn are influenced by the applied field; buffer composition, ionic strength, and pH; capillary surface characteristics; temperature; surfactant type and concentration; and choice of organic modifier and concentration. More often than not these parameters do not vary independently. Some of these aspects will be discussed in the results and discussion section with supporting experimental evidence.

Formal attempts to predict retention in MEKC based on an understanding of the contribution of intermolecular interactions are quite rare. The Kamlet–Taft solvatochromic model, eqn. (1), was applied by Chen *et al.*[9] to estimate the contribution of defined intermolecular interactions to the water–sodium dodecyl sulfate micelle distribution constant of substituted benzene compounds and more extensively by Yang and co-workers[10–13] to characterize the retention properties of aqueous buffer–micelle systems containing the surfactants sodium dodecyl sulfate, sodium cholate, tetradecyltrimethylammonium bromide, and lithium perfluorooctanesulfonate. The product terms ($mV/100$) representing cavity formation, ($s\pi^*$) the

$$\log k = c + mV/100 + s\pi^* + b\beta + a\alpha \quad (1)$$

contribution from dipole-type interactions, and ($b\beta$ and $a\alpha$) the contribution of hydrogen bond acid/base interactions to the retention factor (capacity factor), k. These authors concluded that the type of surfactant has a major influence on selectivity and the size of the migration window but surfactant concentration only influences absolute retention through changes in the phase ratio of the separation system without changing selectivity. For sodium dodecyl sulfate and sodium cholate retention depends mainly on the size of the solute (the micelles are less cohesive than the aqueous buffer) and the basicity of the solute

(water is a strong hydrogen-bond acid favoring retention of hydrogen-bond bases in the buffer). Other factors are of little importance. In the case of lithium perfluorooctanesulfonate the micelles are more cohesive than sodium dodecyl sulfate and sodium cholate, are as strong hydrogen-bond acids as water itself, and weak (by comparison) hydrogen-bond bases. This is a most remarkable result because the perfluorooctanesulfonate group contains no hydrogen-bond acid groups (note proton acidity and hydrogen-bond acidity are unrelated concepts) and the inductive effect of fluorine increases the hydrogen-bond basicity of the perfluorooctanesulfonates, which would be expected to increase their competitive capacity with the aqueous buffer to retain hydrogen-bond acid solutes.[14,15] Tetradecyltrimethylammonium bromide has similar cohesive properties to sodium dodecyl sulfate and sodium cholate but is a significantly stronger hydrogen-bond base than these surfactants and a very weak hydrogen-bond acid, in reasonable agreement with chemical intuition. Thus for all four surfactants differences in their selectivity were assigned to variations in cohesive structure of the micelles and different capacities for hydrogen-bond interactions, with differences in the capacity for dipole-type interactions largely irrelevant.

In many areas of retention modeling in chromatography, for example, stationary phase characterization in gas chromatography[16–19] and retention mapping in column and thin-layer liquid chromatography[20–25] the solvation parameter model has been found preferable to the solvatochromic model. The solute descriptors in the solvation parameter model are clearly free energy related parameters and values for in excess of 2000 solutes are available.[18,22,26–28] The solvation parameter model in a form suitable for characterizing the distribution of solutes between two condensed phases can be set out as indicated by eqn. (2)

$$\log k = c + mV_X/100 + rR_2 + s\pi_2^H + a\alpha_2^H + b\beta_2^H \qquad (2)$$

where V_X is the solute's characteristic volume, R_2 excess molar refraction, π_2^H the ability of the solute to stabilize a neighboring dipole by virtue of its capacity for orientation and induction interactions, and α_2^H and β_2^H are parameters characterizing the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. The system constants in eqn. (2) are unambiguously defined: the *r* constant refers to the difference in capacity of the buffer and micellar phase to interact with solute *n*- or π-electrons; the *s* constant to the difference in capacity of the buffer and micellar phase to take part in dipole–dipole and dipole-induced dipole interactions; the *a* constant is a measure of the difference in hydrogen-bond acceptor basicity of the buffer and micellar phase; the *b* constant is a measure of the difference in hydrogen-bond donor acidity of the buffer and micellar phase; and the *m* constant is a measure of the relative ease of forming a cavity for the solute in the buffer and micellar phase. For any MEKC system the system constants can be obtained using multiple linear regression analysis. Experimentally data are acquired for the observed parameter, log *k*, for a group of solutes of known properties sufficiently varied to define all interactions in eqn. (2) and of sufficient number to establish the statistical validity of eqn. (2).

Abraham *et al.* used the solvation parameter model to characterize the distribution constant of solutes between water and sodium dodecyl sulfate micelles[29] and the retention factor for an oil-in-water type emulsion consisting of 1.4% m/m sodium dodecyl sulfate, 6.49% m/m butan-1-ol, and 0.82% m/m heptane in 100 mm borate–500 mm phosphate buffer at pH 7.[30] The system constants along with the statistics for the fit are assembled in Table 1, row A and L, respectively. Adlard *et al.*[31] have provided a collection of retention factor values for substituted benzene compounds in separation systems containing 40 mm potassium deoxycholate (rows G and H) and 40 mm 3β-glucopyranosyl-5β-cholan-12α-hydroxy-24-oic acid potassium salt (row I) and Herbert and Dorsey[32] for 50 mm sodium

Table 1 Application of the solvation parameter model to micellar separation systems. Surfactants: SDS = sodium dodecyl sulfate; SC = sodium cholate; KDOC = potassium deoxycholic acid; KGDC = potassium salt of 3β-glucopyranosyl-5β-cholan-12α-hydroxy-24-oic acid; LPOS = lithium perfluorooctanesulfonate; and TDTMA = tetradecyltrimethylammonium bromide

Row number	Surfactant/mm	System constants						Statistics*				pH	Reference
		m	*r*	*s*	*a*	*b*	*c*	*R*	*SE*	*F*	*n*		
A	SDS	2.79 (0.07)	0.54 (0.06)	−0.40 (0.07)	−0.13 (0.06)	−1.58 (0.08)	1.20 (0.06)	0.985	0.17	817	187	7	29
B	SDS (50)	2.91 (0.17)	0.31 (0.08)	−0.24 (0.08)	−0.44 (0.08)	−1.87 (0.15)	−1.85 (0.15)	0.994	0.11	397	32	7[a]	32
C	SDS (20)	2.81 (0.09)	0.38 (0.06)	−0.28 (0.08)	−0.16 (0.05)	−1.80 (0.09)	−2.18 (0.08)	0.992	0.07	513	47	7[b]	33
D	SDS (40)	2.82 (0.09)	0.37 (0.06)	−0.33 (0.09)	−0.17 (0.05)	−1.82 (0.09)	−1.80 (0.08)	0.991	0.08	457	47	7[b]	33
E	SC (60)	2.41 (0.12)	0.57 (0.09)	−0.55 (0.11)		−2.45 (0.13)	−1.60 (0.12)	0.984	0.10	328	47	7[b]	33
F	SC (80)	2.63 (0.12)	0.40 (0.08)	−0.60 (0.12)		−2.59 (0.12)	−1.47 (0.11)	0.986	0.10	294	47	7[b]	33
G	KDOC (40)	3.12 (0.18)	0.54 (0.16)	−0.97 (0.20)		−2.47 (0.27)	−1.96 (0.15)	0.992	0.10	135	14	8	31
H	KDOC (40)	2.76 (0.16)	0.67 (0.14)	−0.88 (0.18)		−2.42 (0.25)	−1.80 (0.13)	0.992	0.10	140	14	9	31
I	KGDC (40)	2.89 (0.21)	0.24 (0.12)			−2.97 (0.21)	−2.14 (0.19)	0.990	0.10	142	13	9	31
J	LPOS (40)	2.28 (0.12)	−0.54 (0.08)	0.48 (0.11)	−0.89 (0.07)	−0.60 (0.12)	−2.05 (0.11)	0.979	0.10	189	47	7[b]	33
K	TDTMA (10)	2.76 (0.11)	0.28 (0.07)		0.94 (0.06)	−2.62 (0.10)	−2.09 (0.10)	0.989	0.09	422	47	7[b]	33
L	Emulsion	3.05	0.28	−0.69	−0.06	−2.81	−1.13	0.994	0.09	791	53	7[c]	30

* *R* = correlation coefficient; *SE* = standard error in the estimate; *F* = *F*-statistic; *n* = number of solutes; and numbers in parentheses indicate the standard deviation in the coefficient. [a] 100 mm borate–60 mm phosphate buffer. [b] 50 mm phosphate buffer. [c] 1.4% m/m sodium dodecyl sulfate, 6.49% m/m butan-1-ol and 0.82% m/m heptane in 100 mm borate–500 mm phosphate buffer.

dodecyl sulfate at pH 7 in 100 mm borate–60 mm phosphate buffer (row B). This data we have fitted to the solvation parameter model summarizing the system constants and the statistics for the fit in Table 1. Finally, we had solute descriptors for 47 of the 60 solutes studied by Yang and co-workers[10–13] and Khaledi,[33] discussed earlier in connection with the solvatochromic model, and have fitted this data to the solvation parameter model, summarizing the results in rows C–F, J, and K in Table 1.

In all cases the statistics for the fit of the model to the data are good with that for lithium perfluorooctanesulfonate being the poorest (Table 1, row J). For sodium dodecyl sulfate and the bile salt surfactants (Table 1, row A to I) the cavity contribution (*m* constant) and lone pair–lone pair electron attraction (*r* constant) favor sorption by the micelle while dipole-type interactions (*s* constant) and hydrogen-bond interactions (*a* and *b* constants) favor retention in the aqueous buffer. Sodium dodecyl sulfate, sodium cholate and potassium deoxycholate differ to a small extent in their cohesive energy (ease of cavity formation) but more significantly in their capacity for dipole-type interactions and hydrogen-bond interactions. Sodium cholate and potassium deoxycholate micelles are significantly weaker hydrogen-bond acids than sodium dodecyl sulfate (*b* constant); stronger hydrogen-bond bases (*a* constant); and show a range of capacity for dipole-type interactions in the order potassium deoxycholate < sodium cholate < sodium dodecyl sulfate. The derivatization of potassium deoxycholate with a sugar group (row I) leads to significant changes in selectivity for polar interactions, particularly for interactions of a dipole-type (*s* constant). Lithium perfluorooctanesulfonate (Table 1, row J) is the most cohesive of the micelles (*m* constant), is the most competitive with the aqueous buffer as a hydrogen-bond acid (*b* constant) and for dipole-type interactions (*s* constant), and is a very weak hydrogen-bond base (*a* constant). The negative *r* constant is characteristic of fluoroalkane compounds, in general, representing their lower polarizability compared to alkane chains. Since the perfluorooctanesulfonate group has no available protons for hydrogen bonding we can only speculate that its hydrogen-bond acidity arises from the inductive effect of fluorine on water molecules selectively solvating the sulfonate group. The characteristic feature of tetradecyltrimethylammonium bromide is its strong hydrogen-bond basicity (*a* constant) compared to the other surfactants. Features to note about the data in Table 1 are that (*a*) the solvation parameter model provides a consistent and chemically sensible interpretation of the data, (*b*) the details of the interpretation are different to those of the solvatochromic model used by Yang and co-workers[10–13] (particularly with respect to the relative importance of polar interactions), and (*c*) the identity of the surfactant is the most important factor in controlling selectivity. Differences in the results between the solvation parameter model and solvatochromic model certainly arise because of numerical differences in the solute descriptors (which are derived from different measurement techniques) and also because Yang and co-workers[10–13] used estimates for many descriptors unavailable by experiment.

The results in Table 1 cannot indicate how the selectivity of a surfactant depends on the full range of experimental variables in MEKC nor provide information about the selectivity of other common surfactants for which there is insufficient experimental data available to satisfy the requirements of the solvation parameter model. We will address both of these points in this paper.

Experimental

Sodium tetraborate, sodium phosphate, sodium dodecyl sulfate, cholic acid sodium salt, taurocholic acid sodium salt, taurodeoxycholic acid sodium salt monohydrate, *N*-dodecanoyl-*N*-methyltaurine sodium salt, hexadecyltrimethylammonium bromide, and 0.1 and 1.0 m sodium hydroxide were obtained from Fluka (Gillingham, Dorset, UK). 1-Phenyloctane and the solutes used for retention measurements (Table 2) were obtained from Aldrich (Gillingham, Dorset, UK). Fused silica capillary tubing of 0.05 mm id was obtained from Composite Metal Services (The Chase, Harlow, Worcestershire, UK) and cut to the required length. Windows for on-column detection were prepared by using an electrical ring heater (built in-house) to burn off a small segment of the protective polyimide coating from the fused silica capillary column. All solvents and water were HPLC grade from J. T. Baker (Milton Keynes, Buckinghamshire, UK).

All separations were performed with a Hewlett-Packard ^{3D}CE system (Stockport, Cheshire, UK) with a UV diode array detector and laser jet printer. The fused-silica capillaries were 48.5 cm long (effective length 40 cm) for the determination of system constants and 80.5 cm (effective length 72 cm) for the separations used in the figures. Prior to each separation the capillaries were flushed with 0.1 m sodium hydroxide for 2 min followed by the separation buffer for 5 min. For hexadecyltrimethylammonium bromide additional conditioning was required to obtain stable results. The capillary was flushed with 1.0 m sodium hydroxide for 2 min followed by water for 5 min, and then the normal conditioning cycle was commenced. Each surfactant was studied using a fresh capillary for the determina-

Table 2 Solute descriptors used in the solvation parameter model

	Descriptors				
Solute	$V_X/100$	R_2	π_2^H	α_2^H	β_2^H
Benzene	0.7164	0.610	0.52		0.14
Toluene	0.8573	0.601	0.52		0.14
Ethylbenzene	0.9982	0.613	0.51		0.15
Naphthalene	1.0854	1.340	0.92		0.20
Fluorene	1.3565	1.588	1.03		0.20
Chlorobenzene	0.8388	0.718	0.65		0.07
Iodobenzene	0.9746	1.188	0.82		0.12
Anisole	0.9160	0.708	0.75		0.29
Acetophenone	1.0139	0.818	1.01		0.48
Benzonitrile	0.8711	0.742	1.11		0.33
Nitrobenzene	0.8910	0.871	1.11		0.28
Benzaldehyde	0.8730	0.820	1.00		0.39
Phenyl acetate	1.0730	0.661	1.13		0.54
Methyl benzoate	1.0726	0.733	0.85		0.46
Propyl benzoate	1.3544	0.675	0.80		0.46
Butyl benzoate	1.4953	0.668	0.80		0.46
1,4-Dichlorobenzene	0.9612	0.825	0.75		0.02
3-Nitrotoluene	1.0320	0.874	1.10		0.25
4-Chloroacetophenone	1.1360	0.955	1.09		0.44
1-Nitrobutane	0.8464	0.227	0.95		0.29
1-Nitrohexane	1.1282	0.203	0.95		0.29
Benzyl alcohol	0.9160	0.803	0.87	0.33	0.56
2-Phenylethanol	1.0569	0.811	0.91	0.30	0.64
4-Phenylbutanol	1.3387	0.811	0.90	0.33	0.70
4-Nitrobenzyl alcohol	1.0902	1.064	1.39	0.44	0.62
Acetanilide	1.1133	0.870	1.40	0.50	0.67
Benzenesulfonamide	1.0971	1.130	1.55	0.55	0.80
4-Nitroaniline	0.9910	1.220	1.91	0.42	0.38
N-Methylbenzamide	1.1137	0.950	1.44	0.35	0.73
Phenol	0.7751	0.805	0.89	0.60	0.30
3-Methylphenol	0.9160	0.822	0.88	0.57	0.34
4-*tert*-Butylphenol	1.3387	0.810	0.89	0.56	0.39
4-Phenylphenol	1.3829	1.560	1.41	0.59	0.45
3,5-Dimethylphenol	1.0569	0.820	0.84	0.57	0.36
4-Chloro-3-methylphenol	1.0384	0.920	1.02	0.65	0.23
Methyl 3-hydroxybenzoate	1.1313	0.905	1.40	0.66	0.45
Propyl 4-hydroxybenzoate	1.4131	0.840	1.35	0.69	0.45
2-Naphthol	1.1440	1.520	1.08	0.61	0.40

tion of system constants. Except as stated otherwise measurements were made at 25 °C, +20 kV, and 210 nm. Standard solutions were made up in methanol (1–2 mg ml^{-1}) to which 1-phenyloctane in acetone was added to indicate the micelle migration time. All sample solutions and buffers were filtered through 0.2 μm poly(propylene) syringe filters (Gelman Sciences, Ann Arbor, MI, USA) prior to use. Samples were introduced into the capillary by applying a pressure of 50 mbar for 1–2 s (1 bar = 10^5 Pa).

The retention factor was calculated using eqn. (3) with the migration time of methanol used to determine the electroosmotic flow (t_{eo}) and 1-phenyloctane the migration time of the micelles (t_{mc}) with t_R as the solute migration time

$$k = (t_R - t_{eo})/(1 - t_R/t_{mc})\, t_{eo} \quad (3)$$

The retention factors used with the solvation parameter model to determine the nature of the selectivity of the different micellar systems are summarized in Table 3. The solute descriptors were taken from several sources and are summarized in Table 2 for the reader's convenience.[18,22,26] Multiple linear regression analysis and statistical tests were performed on a Vectra computer (Hewlett- Packard) using the program SPSS/PC+ V3.1 (SPSS, Chicago, IL, USA).

Results and Discussion

A series of experiments were performed with the surfactant sodium cholate to establish the influence of experimental parameters on selectivity. These results are summarized in Table 4 with the boundary conditions based on previous experiences[34] and the necessity to maintain an acceptable separation time and efficiency, and a useful migration window. It is immediately obvious that experimental parameters have only a small influence on selectivity as indicated by the narrow range of system constant values throughout the table. Selectivity, therefore, is controlled primarily by the choice of surfactant. Varying the concentration of the surfactant primarily causes change in the model constant term (*c* constant). Increasing the surfactant concentration results in a general increase in retention due to changes in the phase ratio for the separation system,[2–5,11] but only minor changes in selectivity. Changing the pH (for non-ionized solutes) has a significant effect on the electroosmotic flow, and therefore, the migration time, but over a useful pH range (8, 8.5, 9) little influence on selectivity. The choice of buffer, in this case, sodium phosphate, sodium tetraborate, or a mixture of the two; or buffer concentration (10, 20, 30 mm) has little influence on selectivity. Changes in selectivity accompanying variations in temperature (15, 25,

Table 3 Retention factors used to characterize surfactant selectivity. Sodium phosphate–sodium tetraborate buffer (20 mm), temperature 25 °C, field strength 20 kV, and other conditions indicted in Table 5

	Logarithm of the retention factor (log *k*)						
Solute	Sodium dodecyl sulfate	*N*-Dodecanoyl-*N*-methyl-taurine	Sodium cholate	Sodium deoxycholate	Sodium taurocholate	Sodium tauro-deoxycholate	Hexadecyl-trimethyl ammonium bromide
Benzene	0.044	0.110	−0.210	−0.040	−0.487	−0.294	0.531
Toluene	0.553	0.611	0.387	0.491	−0.033	0.148	0.834
Ethylbenzene	0.997	0.971	0.701	0.845	0.333	0.619	1.221
Naphthalene	1.185	1.014	0.961	1.244	0.588	0.940	1.778
Fluorene	2.136		1.446	2.117	1.205	1.686	
Chlorobenzene	0.618	0.597	0.402	0.595	0.103	0.311	1.023
Iodobenzene	1.036	1.216	0.762	1.085	0.547	0.820	1.545
Anisole	0.260	0.344	−0.037	0.142	−0.386	−0.176	0.436
Acetophenone	0.275	0.056	−0.411	−0.163	−0.569	−0.336	0.105
Benzonitrile	0.032	−0.016	−0.487	−0.160	−0.617	−0.444	0.107
Nitrobenzene	0.143	0.264	−0.262	0.285	−0.408	−0.225	0.396
Benzaldehyde	−0.017	−0.061	−0.500	−0.314	−0.726	−0.490	0.029
Phenyl acetate	0.169	0.022	−0.561	−0.327	−0.696	−0.478	0.181
Methyl benzoate	0.536	0.288	−0.028	0.124	−0.246	−0.012	0.530
Propyl benzoate	1.316	1.046	0.563	0.866	0.385	0.703	1.638
Butyl benzoate	1.801	1.129	0.867	1.298	0.740	1.136	
1,4-Dichlorobenzene	1.076	1.119	0.885	1.138	0.437	0.818	1.628
3-Nitrotoluene	0.637	0.660	0.150	0.368	−0.056	0.160	0.982
4-Chloroacetophenone	0.794	0.667	0.145	0.373	−0.071	0.177	0.803
1-Nitrobutane	−0.180	−0.250	−0.756	−0.520	−0.932	−0.748	−0.017
1-Nitrohexane	0.733	0.658	0.040	0.323	−0.195	0.008	0.961
Benzyl alcohol	−0.268	−0.294	−0.690	−0.517	−0.906	−0.685	−0.131
2-Phenylethanol	0.025	0.038	−0.539	−0.345	−0.723	−0.529	0.138
4-Phenylbutanol	0.752	0.767	0.110	0.278	−0.120	0.080	0.867
4-Nitrobenzyl alcohol	−0.052	0.066	−0.387	−0.233	−0.561	−0.403	0.263
Acetanilide	−0.083	−0.105	−0.458	−0.288	−0.644	−0.489	0.032
Benzenesulfonamide	−0.381	−0.345	−0.808	−0.583	−0.806	−0.690	−0.088
4-Nitroaniline	0.059	0.232	−0.191	−0.191	−0.332	−0.204	0.445
N-Methylbenzamide	−0.191	−0.378	−0.631	−0.360	−0.738	−0.530	0.242
Phenol	−0.306	0.221	−0.257	−0.370	−0.691	−0.552	0.402
3-Methylphenol	0.144	0.370	−0.193	−0.082	−0.398	−0.206	0.790
4-*tert*-Butylphenol	1.082	1.417	0.989	0.999	0.638	0.757	1.935
4-Phenylphenol	1.412	1.505	1.016	1.207	0.789	0.996	
3,5-Dimethylphenol	0.504	0.769	0.130	0.234	−0.091	0.057	1.224
4-Chloro-3-methylphenol	0.803	1.221	0.596	0.646	0.354	0.475	
Methyl 3-hydroxybenzoate	0.324	0.453	−0.028	0.163	−0.274	−0.088	0.799
Propyl 4-hydroxybenzoate	1.021	1.213	0.659	0.743	0.391	0.528	1.913
2-Naphthol	0.914	1.345	0.567	0.650	0.355	0.542	1.636

35 °C) are again small indicating that, at least for sodium cholate, this is not a particularly useful parameter for selectivity optimization. Adding methanol to the buffer in amounts from 1 to 10% (v/v) has a small influence on the ease of cavity formation (*m* constant) but more significantly results in a reduction of the hydrogen-bond acidity of the aqueous buffer (either due to alterations of the capacity of the micellar phase or buffer solution for hydrogen-bond interactions). The presence of organic solvent will also affect the critical micelle concentration of the surfactant with a possible change in the phase ratio[35,36] but this must be small for the systems studied here, given the narrow range of values for the model constant. Surprisingly, the choice of organic solvent (5% v/v), whether methanol, acetonitrile, tetrahydrofuran, or propan-2-ol, seems to be virtually without influence on the selectivity, in complete contrast to reversed-phase liquid chromatography, often used as an analogous system to explain separation characteristics in MEKC. It is possible that low-molecular-mass solvents are poorly taken up by the micelles and function primarily as a diluent of the buffer, where within the concentration range studied, they moderate the characteristic solvophobic properties of water more so than modify the selectivity of the micellar phase. Solubility limitations and the unfavorable influence of organic solvents on the electroosmotic flow prevent exploring the mechanism of this observation at significantly higher concentrations of organic solvent. The latter conclusions are roughly corroborated by the studies of Garcia *et al.*[37] on the solute–micelle association constants for a group of benzene derivatives and polycyclic aromatic hydrocarbons using sodium dodecyl sulfate as surfactant. They concluded that the association constants were not significantly influenced by the concentration [5 or 10 mm] or type of organic buffer [2-(*N*-cyclohexylamino)ethanesulfonic acid or sodium acetate], or by different concentrations of propan-1-ol (3% v/v) and butan-1-ol (1, 3, 5% v/v). These studies were performed at pH 9 and 10, at which many of the phenols in their data set would be significantly ionized, leaving too few solutes for a robust fit of the solvation parameter model to their data.

Table 4 Influence of experimental variables on the selectivity of the surfactant sodium cholate in MEKC. Field strength kV. Solutes (n = 40) are indicated in Table 2*

	System constants						Statistics		
Range	*m*	*r*	*s*	*a*	*b*	*c*	*R*	*SE*	*F*
(1) *Sodium phosphate–sodium tetraborate buffer (20 mM); pH 8; temperature 25 °C; vary concentration of surfactant—*									
50 mm	2.59	0.65	−0.47		−2.27	−2.11	0.985	0.11	275
	(0.13)	(0.09)	(0.10)		(0.13)	(0.13)			
75 mm	2.45	0.63	−0.47		−2.29	−1.71	0.983	0.11	241
	(0.12)	(0.08)	(0.09)		(0.13)	(0.12)			
125 mm	2.39	0.48	−0.46		−2.14	−1.34	0.986	0.10	281
	(0.10)	(0.07)	(0.08)		(0.12)	(0.10)			
(2) *Sodium phosphate–sodium tetraborate buffer (20 mM); surfactant concentration (75 mM); temperature 25 °C; vary pH—*									
pH 8.5	2.59	0.61	−0.47		−2.31	−1.90	0.987	0.10	308
	(0.11)	(0.07)	(0.08)		(0.12)	(0.11)			
pH 9.0	2.45	0.57	−0.41		−2.26	−1.77	0.982	0.11	229
	(0.12)	(0.08)	(0.09)		(0.13)	(0.12)			
(3) *Buffer concentration (20 mM); surfactant concentration (75 mM); pH 8; temperature 25 °C; vary buffer type—*									
Sodium phosphate	2.52	0.59	−0.48		−2.26	−1.79	0.988	0.10	343
	(0.10)	(0.06)	(0.07)		(0.11)	(0.10)			
Sodium tetraborate	2.58	0.63	−0.46		−2.30	−1.93	0.982	0.11	229
	(0.11)	(0.07)	(0.08)		(0.13)	(0.11)			
(4) *Sodium phosphate–sodium tetraborate buffer; surfactant concentration (75 mM); pH 8; temperature 25 °C; vary buffer concentration—*									
10 mm	2.43	0.60	−0.50		−2.23	−1.63	0.984	0.11	251
	(0.11)	(0.07)	(0.08)		(0.13)	(0.11)			
30 mm	2.49	0.60	−0.50		−2.29	−1.65	0.986	0.10	289
	(0.11)	(0.07)	(0.08)		(0.12)	(0.11)			
(5) *Sodium phosphate–sodium tetraborate buffer (20 mM); surfactant concentration (75 mM); pH 8; vary temperature—*									
15 °C	2.63	0.61	−0.41		−2.49	−1.87	0.982	0.13	220
	(0.13)	(0.08)	(0.10)		(0.15)	(0.13)			
35 °C	2.38	0.58	−0.49		−2.05	−1.75	0.985	0.10	278
	(0.10)	(0.07)	(0.08)		(0.12)	(0.10)			
(6) *Sodium phosphate–sodium tetraborate buffer (20 mM); surfactant concentration (75 mM); pH 8; temperature 25 °C; vary volume of methanol (v/v)—*									
1%	2.50	0.60	−0.48		−2.26	−1.71	0.985	0.11	261
	(0.11)	(0.07)	(0.08)		(0.13)	(0.11)			
5%	2.34	0.57	−0.41		−2.07	−1.74	0.987	0.09	309
	(0.09)	(0.06)	(0.07)		(0.11)	(0.10)			
10%	2.23	0.56	−0.36		−1.95	−1.79	0.986	0.09	280
	(0.09)	(0.06)	(0.07)		(0.11)	(0.09)			
(7) *Sodium phosphate–sodium tetraborate buffer (20 mM); surfactant concentration (75 mM); pH 8; temperature 25 °C; vary organic solvent identity at 5% (v/v)—*									
Acetonitrile	2.30	0.52	−0.36		−2.08	−1.72	0.987	0.09	315
	(0.09)	(0.06)	(0.07)		(0.11)	(0.09)			
Tetrahydrofuran	2.08	0.49	−0.39		−2.00	−1.52	0.986	0.09	230
	(0.09)	(0.06)	(0.07)		(0.11)	(0.09)			
Propan-2-ol	2.27	0.56	−0.40		−2.03	−1.68	0.986	0.09	299
	(0.09)	(0.06)	(0.07)		(0.11)	0.09)			

* Definition of statistics as in Table 1. Numbers in parentheses indicate to standard deviation in the coefficient.

In terms of selectivity optimization the above experiments indicate that varying the choice of surfactant is more likely to result in significant changes in selectivity for neutral solutes than fine tuning the operational parameters suitable for the separation. Table 5 summarizes the system constants for seven commonly used surfactants in MEKC. The group of bile salt surfactants, sodium cholate, sodium deoxycholate, sodium taurocholate, and sodium taurodeoxycholate, are most similar to each other, while as a group different to the other surfactants in Table 5. Notably, solute hydrogen-bond acid interactions do not contribute to retention (*a* constant is statistically insignificant). Choosing surfactants from within this group can only be expected to provide small changes in selectivity, most notably for solutes with a significant capacity as hydrogen-bond bases. The migration order for the test mixture benzenesulfonamide, 2-phenylethanol, 3-methylphenol, anisole, methyl 3-hydroxybenzoate, 3-nitrotoluene, ethylbenzene, propyl benzoate, and naphthalene is the same for all four bile salts. Separation is complete using sodium taurodeoxycholate, Fig. 1; while anisole and methyl 3-hydroxybenzoate comigrate using sodium cholate, Fig. 2; ethylbenzene and propyl benzoate are incompletely separated using sodium deoxycholate, Fig. 3; and anisole and

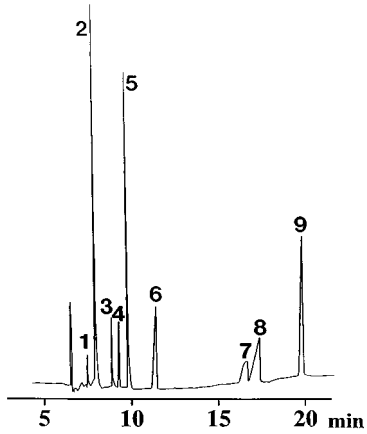

Fig. 1 Separation of a test mixture by MEKC using 50 mm sodium taurodeoxycholate in a 20 mm sodium phosphate–sodium borate buffer, pH 8, 35 °C, 30 kV, and 80.5 cm capillary (effective length = 72 cm). Peak identification: 1, benzenesulfonamide; 2, 2-phenylethanol; 3, 3-methylphenol; 4, anisole; 5, methyl 3-hydroxybenzoate; 6, 3-nitrotoluene; 7, ethylbenzene; 8, propyl benzoate; and 9, naphthalene.

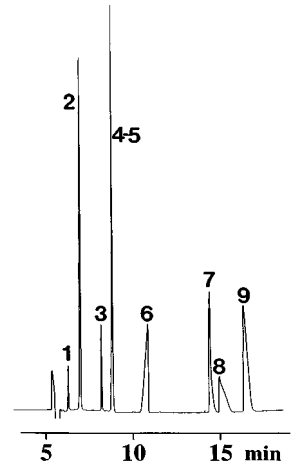

Fig. 2 Separation of a test mixture by MEKC using 75 mm sodium cholate. Other conditions and peak identifications as for Fig. 1.

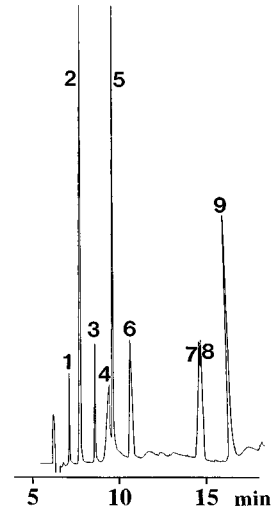

Fig. 3 Separation of a test mixture by MEKC using 75 mm sodium deoxycholate. Other conditions and peak identifications as for Fig. 1.

Table 5 Selectivity of different surfactants at 25 °C in a 20 mm sodium phosphate–sodium tetraborate buffer and field strength 20 kV. The retention factors used to fit the model are given in Table 3

Surfactant	Concentration/mm	pH	System constants						Statistics*			
			m	*r*	*s*	*a*	*b*	*c*	*R*	*SE*	*F*	*n*
Sodium dodecyl sulfate	50	8	2.99 (0.07)	0.46 (0.05)	−0.44 (0.05)	−0.30 (0.05)	−1.88 (0.08)	−1.82 (0.07)	0.994	0.07	569	40
N-Dodecanoyl-*N*-methyltaurine	50	8	3.07 (0.09)	0.72 (0.06)	−0.50 (0.07)	0.22 (0.06)	−2.58 (0.10)	−2.01 (0.09)	0.992	0.08	338	39
Sodium cholate	75	8	2.45 (0.12)	0.63 (0.08)	−0.47 (0.09)		−2.29 (0.13)	−1.71 (0.12)	0.983	0.11	241	40
Sodium deoxycholate	75	8	2.67 (0.11)	0.66 (0.08)	−0.47 (0.09)		−2.47 (0.13)	−1.69 (0.12)	0.986	0.11	286	40
Sodium taurocholate	50	8	2.43 (0.09)	0.60 (0.07)	−0.34 (0.07)		−2.06 (0.10)	−2.10 (0.09)	0.989	0.09	377	40
Sodium taurodeoxycholate	50	8	2.62 (0.09)	0.67 (0.06)	−0.45 (0.07)		−2.17 (0.10)	−1.99 (0.09)	0.991	0.09	430	40
Hexadecyltrimethylammonium bromide	50	7	3.40 (0.10)	0.61 (0.06)	−0.55 (0.07)	0.58 (0.06)	−3.08 (0.10)	−1.67 (0.11)	0.993	0.08	436	36

* Definition of statistics as in Table 1.

3-methylphenol comigrate using sodium taurocholate, Fig. 4. Peak position in the chromatogram is the result of a balance of intermolecular interactions combined with size differences. For example, the comigration of anisole and methyl 3-hydroxybenzoate using sodium cholate results largely from the fact that the larger contribution to retention from the cavity term ($mV_X/100$) for methyl 3-hydroxybenzoate is compensated by its stronger interactions of a dipole-type ($s\pi_2^H$) and hydrogen-bonding ($b\beta_2^H$) with the aqueous electrolyte compared to anisole resulting in coincidental retention factors. Separation using the other bile salt surfactants results from the fact that the compensating balance of interactions is lost to the extent that separation is now possible. A powerful feature of the solvation parameter model is its usefulness in dissecting retention information into intermolecular interactions that provides a fundamental insight into retention mechanisms that is absent from simply noting peak positions in a series of chromatograms. The latter approach, which is common practice in chromatography, presupposes that dominant intermolecular interactions can be associated with individual compounds, which is not a tenable case as indicated by the varied compounds in Table 2.

Sodium dodecyl sulfate has different selectivity to the bile salt surfactants, Table 5. It is slightly less cohesive (larger *m* constant) but more importantly, it is a significantly weaker hydrogen-bond base (negative *a* constant) and stronger hydrogen-bond acid (more competitive with water for hydrogen-bond acid interactions; smaller negative *b* constant). Compared to the bile salt surfactants solutes with a significant capacity for hydrogen-bond interactions will be most affected in their migration order. This is notable in the relative retention for the test mixture, Fig. 5, where peak reversal for naphthalene and propyl benzoate also occurs compared with the bile salt surfactants. The greater hydrogen-bond acidity of sodium dodecyl sulfate competing more effectively with water for the retention of the hydrogen-bond base propyl benzoate combined with a more favorable cavity term largely explains the change in peak order observed.

Sodium *N*-dodecanoyl-*N*-methyltaurine is a significantly stronger hydrogen-bond base (positive *a* constant) and weaker hydrogen-bond acid (large negative *b* constant) than sodium dodecyl sulfate. The most notable difference in separation properties between the sodium salt of *N*-dodecanoyl-*N*-methyltaurine and the bile salts and sodium dodecyl sulfate is again for those solutes with a significant capacity for hydrogen-bond interactions. These differences are reflected in the peak reversal of 3-methylphenol and anisole for the test mixture compared to the bile salts and sodium dodecyl sulfate, Fig. 6.

The selectivity of hexadecyltrimethylammonium bromide is most like the sodium salt of *N*-dodecanoyl-*N*-methyltaurine, except that it is considerably less cohesive (largest *m* constant of the surfactants in Table 5), it has the lowest capacity for dipole-

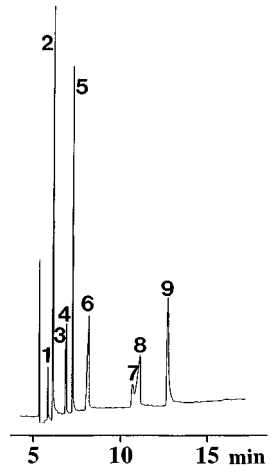

Fig. 4 Separation of a test mixture by MEKC using 50 mm sodium taurocholate. Other conditions and peak identifications as for Fig. 1.

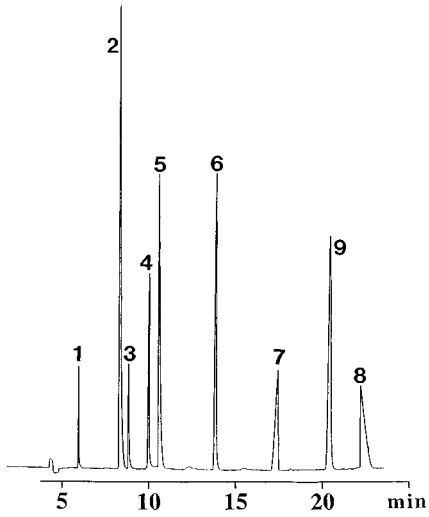

Fig. 5 Separation of a test mixture by MEKC using 50 mm sodium dodecyl sulfate. Other conditions and peak identifications as for Fig. 1.

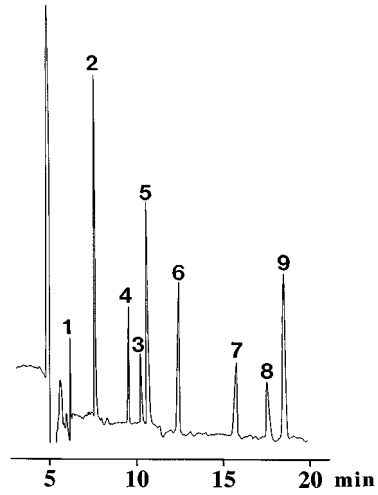

Fig. 6 Separation of a test mixture by MEKC using 50 mm sodium *N*-dodeconyl-*N*-methyltaurine. Other conditions and peak identifications as for Fig. 1.

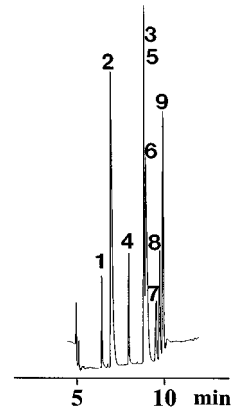

Fig. 7 Separation of a test mixture by MEKC using 50 mm hexadecyltrimethylammonium bromide. Other conditions and peak identifications as for Fig. 1 except that the potential was reversed.

type interactions (largest negative *s* constant), it is the strongest hydrogen-bond base (largest *a* constant), and the weakest hydrogen-bond acid (largest negative *b* constant). As well as a higher general retention of solutes of low polarity, the greatest difference in migration order compared to the other surfactants in Table 5, is expected for hydrogen-bond forming solutes. The separation of the test mixture is shown in Fig. 7.

From Table 5 a choice of one of the bile salts and the three remaining surfactants provides a reasonable range of micellar phases for selectivity optimization with a much greater variation of system constants than was observed for the optimization of experimental conditions in Table 4. To this list should be added lithium perfluorooctanesulfonate from Table 1, whose properties are quite different to those of the surfactants in Table 5. It is noteworthy that the selectivity range for dipole-type interactions is the least satisfactory (*s* constant) and that differences in cohesive properties are quite shallow.

Conclusions

The system constants in Tables 1 and 5 can act as a guide to the selection of surfactants for selectivity optimization in MEKC. Hexadecyltrimethylammonium bromide has complementary selectivity to the other surfactants in Table 5 but provides only a small migration window, which would limit its use for separating complex mixtures. The surfactants exhibit a reasonable range of selectivity but by no means a comprehensive range, such that the introduction of novel surfactants with complementary properties would be welcome. The solvation parameter model provides a basis for characterizing the selectivity of surfactants for MEKC and can be used to identify new surfactants with properties that differ from those indicated here.

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