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Recommendations for the determination of selectivity in micellar electrokinetic chromatography

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Abstract

A general approach for characterizing selectivity in micellar electrokinetic chromatography based on the solvation parameter model is recommended. Individual surfactants are characterized by their cohesion and capacity for polar interactions indicated as lone pair–lone pair electron attraction, dipole-type interactions, and hydrogen bond acidity and basicity. The statistical and chemical validity of the solvation parameter model requires that retention data are determined for a collection of 20–40 varied solutes with a wide range of retention factors and that clustering of values and cross-correlation among the solute descriptors are absent. Since micelles are interfacial solvents with properties that can vary with changes in their external environment (buffer composition, concentration, pH, temperature, etc.) a generic set of experimental conditions are recommended for the measurement of anionic surfactant selectivity under standard conditions. The system constants used to characterize surfactant selectivity are calculated for 12 common surfactants used in micellar electrokinetic chromatography. Of these surfactants sodium dodecyl sulfate, sodium cholate, lithium perfluorooctanesulfonate, sodium N-dodecanoyl-N-methyltaurine and tetradecyltrimethylammonium bromide are identified as providing a useful range of selectivity differences for methods development in micellar electrokinetic chromatography.

Keywords: Selectivity; Solvation parameter model

1. Introduction

Neutral solutes are separated in micellar electrokinetic chromatography (MEKC) if they have different distribution constants between the psuedostationary phase (charged micelles) and the bulk electrolyte. The high efficiency (ca. >200 000 theoretical plates/m) and flexibility of adjusting selectivity by using different surfactants or mixtures of surfactants [1–9], by adding different complexing agents (e.g., cyclodextrins, urea, chiral additives, etc.) [10–12] or organic solvents [13–15] to the separation buffer, has resulted in an extensive number of practical applications [11,16,17]. It is now generally accepted, that in the absence of additives or organic solvent the choice of surfactant is the most important consideration in optimizing selectivity, although formal models of retention, which must underpin any theoretical understanding of methods development in MEKC, have been slow to develop [18]. The exception is the use of solvatochromic and solvation parameter models which will be discussed here.

The solvatochromic and solvation parameter models are based on a cavity model of solvation [19–22]. To transfer a solute from one condensed phase to another proceeds by forming a cavity in the acceptor phase of a suitable size to hold the solute, reorganization of the solvent molecules around the solute cavity, and the set up of intermolecular interactions of a solute–solvent type. The reverse

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process takes place in the donor phase with the free energy for the transfer defining the solute distribution constant for the system. The striking feature of the solvatochromic and solvation parameter models is that the solvation process is described in terms of fundamental intermolecular interactions with solute or solvent properties determined from spectroscopic measurements (usually) in the case of the solvatochromic model and from equilibrium processes in the case of the solvation parameter model. These processes are fairly involved and require standard conditions as outlined in detail elsewhere [22–24]. If a varied and well characterized group of solutes with a known capacity for specific intermolecular interactions are separated in a chromatographic system then by modeling their retention characteristics it is possible to define the complementary capacity of the chromatographic system for the defined intermolecular interactions. This is the basis of system characterization for selectivity optimization and the prediction of retention in chromatography that will be applied to MEKC in this paper.

The choice of an appropriate model is the first step. We prefer the solvation parameter model over the solvatochromic model because all solute descriptors are derived from, or related to, free energy processes. The Kamlet–Taft solvatochromic model was employed by Chen et al. [25], Yang et al. [26–29] and Muijselaar et al. [30] to determine the selectivity of a number of surfactant systems in MEKC. To enable the widest possible characterization of surfactant properties with a single model we have recalculated the data presented in the above papers in terms of the solvation parameter model and will present that data here. Since there are numerical differences in the values for the solute descriptors and slight differences in form for the two models, exact agreement in quantitative aspects cannot be expected, although radical discrepancies in overall trends predicted by both models are rare.

The solvation parameter model is set out below in the form suitable for use in MEKC

$$\mathrm{SP} = c + mV_\mathrm{X} + rR_2 + s\pi_2^\mathrm{H} + a\Sigma\alpha_2^\mathrm{H} + b\Sigma\beta_2^0. \qquad (1)$$

SP is some experimentally observed retention property such as the retention factor ($\log k$) or a distribution constant ($\log K$). The solute descriptors are McGowan's characteristic volume V_X (in $\mathrm{cm}^3\ \mathrm{mol}^{-1}/100$), excess molar refraction R_2 (in $\mathrm{cm}^3/10$), π_2^H the ability of the solute to stabilize a neighboring dipole by virtue of its capacity for orientation and induction interactions, and $\Sigma\alpha_2^\mathrm{H}$ and $\Sigma\beta_2^0$ are the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. For distribution systems in which one phase is water, some solutes exhibit variable hydrogen-bond basicity depending on the solubility of water in the counter phase [31]. This is true of compounds such as anilines, pyridines and sulfoxides resulting in the use of two descriptors for these solutes, $\Sigma\beta_2^\mathrm{H}$ and $\Sigma\beta_2^0$. The intended application indicates which solute descriptor is most appropriate. For MEKC with an aqueous buffer $\Sigma\beta_2^0$ is the solute descriptor usually chosen. The subscript 2 identifies the solute descriptors as values applicable to infinite dilution in a solvent other than themselves (sometimes called monomer values) and should be distinguished from similar symbols with the subscript 1 representing solvent values (appropriate for interactions with like solvent molecules).

The solute's characteristic volume is calculated from its structure by summing the characteristic atomic volumes for each atom and subtracting a fixed amount for each bond [21,32]. It is divided by 100 for rough scaling with the other solute descriptors. The solute's excess molar refraction is calculated from the refractive index and characteristic volume as the difference in molar refraction of the solute and an *n*-alkane of identical volume [33]. It is divided by 10, again, to achieve rough scaling with the other solute descriptors. The excess molar refraction can generally be calculated for most compounds since there are several methods of estimating the refractive index of a compound from fragmental constants and the general relationship between the characteristic volume and properties of the *n*-alkanes are well established. The other solute descriptors must be determined by experiment using chromatographic or liquid–liquid distribution systems [22,23,31,34] or estimated using various parameter estimates and computational approaches [22,23,35–38]. Since solute descriptors are available for some 2000 compounds or more there are usually few problems in identifying a sufficient number and variety of solutes for characterizing chromatographic

systems used to separate low-molecular-mass compounds.

The system constants in Eq. (1) are defined by their complementary interactions with the solute descriptors. The *r* constant determines the difference in capacity of the micelles and mobile phase (separation buffer and additives) to interact with solute n- or π-electrons; the *s* constant to the difference in capacity of the micelles and mobile phase to take part in dipole–dipole and dipole–induced dipole interactions; the *a* constant is a measure of the difference in hydrogen-bond basicity of the micelles and the mobile phase; the *b* constant is a measure of the difference in hydrogen-bond acidity of the micelles and mobile phase; and the *m* constant is a measure of the relative ease of cavity formation and general dispersion interactions for the solute in the micelles and mobile phase. For any separation system, the system constants can be obtained by multiple linear regression analysis of experimental SP values acquired for a group of varied solutes with known descriptors.

2. Selecting appropriate solute descriptors to determine system constants in MEKC

The main criteria in the selection of solute descriptors are that the descriptors should be of sufficient number and variety to establish the statistical and chemical validity of the model, there should be an absence of significant cross-correlation among the chosen set of descriptors, and clustering of individual descriptor values should be avoided. From a practical point of view, the solutes should have a reasonable absorbance between 200–250 nm for convenient detection, since absorption detection is the common mode of detection in MEKC. A collection of solute descriptors suitable for system characterization in MEKC are assembled in Table 1; other values, if needed, can be found in Refs. [21–23,31,39,40].

If all interactions in the solvation parameter model contribute to retention it has been argued that a reasonable fit to the model can be obtained with a minimum of nine solutes [41,42]. Mathematically, a minimum number of seven solutes is needed to do multiple linear regression for the six unknowns (five system constants and the intercept). For statistical soundness three varied values for each solute descriptor and the intercept is a reasonable minimum, but since individual solutes express several interactions simultaneously, the minimum number of required solutes can be safely reduced from about 18 to 9. Here it is presupposed that there is no significant variation in the error associated with individual measurements, which we will show later is untrue in MEKC, so to aim for the minimum number of solutes for system characterization seems unwise. It is common practice to overdetermine the statistical requirements of Eq. (1) to obtain an exhaustive fit, that is a fit which shows little variation in the system constants as small groups of solutes selected at random are deleted. This can usually be achieved using 20 to 40 varied solutes.

A varied collection of solute descriptors may be assembled and found to be inadequate if significant cross-correlation exists between descriptors or the numerical values for the descriptors are clustered. Cross-correlation results from the unintentional correlation between descriptors and loss of capacity of the multiple linear regression algorithm to distinguish between the correlated descriptors. Clustering is easily identified by inspection. Many solute descriptors have similar values (particularly compounds in a homologous series) which can produce a vary narrow range of values for a particular solute descriptor and diminished accuracy in the determination of the complementary system constants. This is most common for $\Sigma\alpha_2^H$ since the number of solutes with significant hydrogen-bond acidity is limited to begin with, and restricted by the requirement that the solute be neutral at all pH values to be used for retention measurements. To obtain adequate electroosmotic flow with fused silica capillary columns a basic pH is commonly used rendering many phenols and carboxylic acids unsuitable solutes due to ionization. At acid pH protonation of amines has also to be considered.

3. Selecting the dependent variable to determine system constants in MEKC

The dependent variable is the free energy related property determined by the experiment for each of the solutes used to build the model. For MEKC this

Table 1
Solute descriptors used in the solvation parameter model for surfactant characterization in MEKC

Solute	Descriptors				Solute	Descriptors				
	V_X	R_2	π_2^H	$\Sigma\beta_2^0$		V_X	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$
Benzene	0.7164	0.610	0.52	0.14	1-Nitrobutane	0.8464	0.227	0.95		0.29
Toluene	0.8573	0.601	0.52	0.14	1-Nitrohexane	1.1282	0.203	0.95		0.29
Ethylbenzene	0.9982	0.613	0.51	0.15	Benzyl Cyanide	1.0120	0.751	1.15		0.45
Propylbenzene	1.1391	0.604	0.50	0.15	Azobenzene	1.3790	1.959	1.13		0.18
Butylbenzene	1.2800	0.600	0.51	0.15	Benzofuran	0.9050	0.888	0.83		0.15
Naphthalene	1.0854	1.340	0.92	0.20	Caffeine	1.3632	1.500	1.60		1.33
1-Methylnaphthalene	1.2263	1.344	0.90	0.20	Quinoline	1.044	1.268	0.97		0.54
Biphenyl	1.3242	1.360	0.99	0.22	1-Nitronaphthalene	1.2596	1.600	1.51		0.29
Fluorene	1.3565	1.588	1.03	0.20	Benzyl alcohol	0.9160	0.803	0.87	0.33	0.56
Phenanthrene	1.4540	2.055	1.29	0.26	2-Phenylethanol	1.0569	0.811	0.91	0.30	0.64
Anthracene	1.4540	2.290	1.34	0.26	4-Phenylbutanol	1.3387	0.811	0.90	0.33	0.70
Fluorobenzene	0.7341	0.477	0.57	0.10	4-Nitrobenzyl alcohol	1.0902	1.064	1.39	0.44	0.62
Chlorobenzene	0.8388	0.718	0.65	0.07	Acetanilide	1.1133	0.870	1.40	0.50	0.67
Bromobenzene	0.8914	0.882	0.73	0.09	Benzenesulfonamide	1.0971	1.130	1.55	0.55	0.80
Iodobenzene	0.9746	1.188	0.82	0.12	Aniline	0.8162	0.955	0.96	0.26	0.50
Anisole	0.9160	0.708	0.75	0.29	N-Methylaniline	0.9571	0.948	0.90	0.17	0.43
Acetophenone	1.0139	0.818	1.01	0.48	4-Nitroaniline	0.9910	1.220	1.91	0.42	0.38
Benzonitrile	0.8711	0.742	1.11	0.33	4-Chloroaniline	0.9390	1.060	1.13	0.30	0.35
Nitrobenzene	0.8910	0.871	1.11	0.28	N-Methylbenzamide	1.1137	0.950	1.44	0.35	0.73
Benzaldehyde	0.8730	0.820	1.00	0.39	Cortisone	2.7550	1.960	3.50	0.35	1.84
Phenyl acetate	1.0730	0.661	1.13	0.54	Hydrocortisone	2.7980	2.030	3.49	0.70	1.87
Methyl benzoate	1.0726	0.733	0.85	0.46	Corticosterone	2.7390	1.860	3.43	0.40	1.63
Propyl benzoate	1.3544	0.675	0.80	0.46	Phenol	0.7751	0.805	0.89	0.60	0.30
Butyl benzoate	1.4953	0.668	0.80	0.46	3-Methylphenol	0.9160	0.822	0.88	0.57	0.34
1,2-Dichlorobenzene	0.9612	0.872	0.78	0.04	4-Methylphenol	0.9160	0.820	0.87	0.57	0.32
1,4-Dichlorobenzene	0.9612	0.825	0.75	0.02	4-Ethylphenol	1.0570	0.800	0.90	0.55	0.36
1,2,3,4-Tetrachlorobenzene	1.2060	1.180	0.92	0.00	4-*tert*.-Butylphenol	1.3387	0.810	0.89	0.56	0.39
2,3,4,5-Tetrachlorobenzene	1.2060	1.160	0.85	0.00	4-Phenylphenol	1.3829	1.560	1.41	0.59	0.45
3-Nitrotoluene	1.0320	0.874	1.10	0.25	3,5-Dimethylphenol	1.0569	0.820	0.84	0.57	0.36
4-Nitrotoluene	1.0320	0.870	1.11	0.28	4-Chloro-3-methylphenol	1.0384	0.920	1.02	0.65	0.23
4-Choroacetophenone	1.1360	0.955	1.09	0.44	Methyl 3-hydroxybenzoate	1.1313	0.905	1.40	0.66	0.45
Ethylphenylketone	1.1550	0.804	0.95	0.51	Propyl 4-hydroxybenzoate	1.4131	0.860	1.35	0.69	0.45
Propylphenylketone	1.2960	0.797	0.95	0.50	2-Naphthol	1.1440	1.520	1.08	0.61	0.40
Butylphenylketone	1.4370	0.795	0.95	0.50	Indole	0.9460	1.200	1.12	0.44	0.31

will normally be a distribution constant or the retention factor. Both must be used and interpreted with some caution. The micelle has too small an aggregation number to be considered as a phase in the usual sense and yet normally contains too many surfactant molecules to be considered a chemical species [43,44]. It is this dichotomy that makes an exact theory of solubilization by micelles difficult. Technically, bulk thermodynamics should not apply to solutes partitioning into small aggregates, since these phases are interfacial phases with large surface to volume ratios. Surface effects could exert a considerable influence on retention and solubility properties may depend on the distance from the interface. Notwithstanding these objections considerable progress has been made in understanding the solubility properties (and, therefore, retention characteristics in chromatographic systems) using general theories based on a pseudophase model or mass action equilibrium model.

There is no uniformity in the definition of equilibrium constants used to represent the solubilization of solution components by surfactant micelles. Treating the micelle as a pseudophase in which the surfactant and solubilized solute reside, one may define a dimensionless partition coefficient ratio by

$$K_X = X_M / X_W \quad (2)$$

where X_M represents the mole fraction of the solute in the micelle and X_W the mole fraction of the solute in the extramicellar bulk solvent. Other concentration units may be used in place of mole fraction, including solute molarity, although this requires knowledge of the partial molar volume of the micelle. Implicit in this use of K_X is the supposition that the solute is distributed homogeneously within the micelle, forming an ideal mixture with the surfactant.

In keeping with the mass action model for incorporation of a solute into the micelle a new equilibrium constant can be written for the process

$$S_W + M \leftrightarrow S_M \quad (3)$$

$$K_M = [S_M]/[S_W][M] \quad (4)$$

where $[S_M]$ is the total concentration of micelle incorporated solute, $[S_W]$ the equilibrium concentration of solute in the intermicellar aqueous phase, and [M] the total concentration of micelles present in solution. K_M represents the binding constant for a single molecule of solute transferred from the bulk aqueous phase into a single micelle. From a practical standpoint, the use of K_M to describe solute incorporation suffers from the inconvenience of requiring a knowledge of the concentration of the micelles, which in turn requires an accurate value for the average aggregation number, that may not be available. As a consequence, the more common form of representation is K_S

$$K_S = [S_M]/[S_W][D] \quad (5)$$

where [D] is the concentration of micellized surfactant.

A minor correction is required to convert any of the distribution constants into thermodynamic constants if the organic solute is not ideal (Henry's law sense) in the aqueous phase. It is rarely justifiable to assume that the equilibrium constant will remain constant as the intramicellar composition varies throughout wide ranges. The maximum additive concentration method is only capable of determining the distribution constant of a solute into the micelle under conditions where the micelle contains the maximum mole fraction of solute. Other methods lead to the determination of the distribution constant in the opposite region, namely in the Henry's law limit, where $X \rightarrow 0$.

The different binding constants are related by:

$$K_X = 55.5 K_S \quad (6)$$

$K_M = (AN)K_S$ where (AN) is the average aggregation number. (7)

The retention factor in MEKC is calculated by Eq. (8)

$$k = (t_R - t_{eo})/(1 - t_R/t_{mc})t_{eo} \quad (8)$$

where t_R is the solute retention time, t_{eo} the retention time of an unretained solute dependent on the electroosmotic velocity of the mobile phase, and t_{mc} the migration time of a solute totally incorporated in the micelle phase. The determination of t_{eo} and t_{mc} is somewhat subjective in that solutes need to be identified by intuition with the appropriate prescribed properties. Either methanol or formamide is often chosen to determine t_{eo} and sudan III, dodecaphenone, or phenyloctane to determine t_{mc}, alternatively, various iteration procedures employing a homologous series of compounds have been suggested [45]. For mixed surfactant micelles the formation of micelles with different compositions can make an accurate determination of t_{mc} difficult or impossible [46]. In all cases we end up with a presumptive measure of the retention factor that must be taken on trust. The retention factor is related to the distribution constants through Eqs. (6), (7) and (9)

$$\log k = \log K_X + \log \phi \quad (9)$$

where ϕ is the assumed phase ratio for the separation system. A comparison of Eq. (9) and Eq. (1) shows that the system constants are independent of whether $\log k$ or $\log K$ is used for the analysis (assuming that $\log K$ is determined by a method that approximates infinite dilution conditions) and only the equation constant (c term) will be different. From a practical point of view solutes with t_R close in value to either t_{eo} or t_{mc} contain significantly larger errors than those for midrange values [47] and data sets with a large number of very small or very large retention factor values may fail to produce statistically sound models. Ideally, the selected solutes should

provide a range of retention factor values spanning the migration window with the minimum number of values of $k \rightarrow 0$ and $k \rightarrow \infty$.

4. Some examples of model fits using small data sets

Some of the difficulties in obtaining an adequate model for the interpretation of surfactant selectivity in MEKC can be illustrated by analysis of some small data sets. At the outset it must always be considered that a small data set may not be representative of large data sets. Chen et al. [25] used MEKC to determine the micelle–buffer distribution constant (K_X) for eleven solutes in a series of sodium dodecyl sulfate buffers at pH 8.5 containing 25 m*M* sodium borate, Table 2. Given the limited possibilities of such a small data set, inspection of the solute descriptors and distribution constants in Table 2 indicates that the solutes seem well chosen, the range of individual values for the descriptors and the distribution constant is reasonable, and cross-correlation is not a problem. By multiple linear regression the following model is obtained

$$\log K_X = 2.45(\pm 0.26)V_X + 0.50(\pm 0.15)R_2 - 0.53(\pm 0.17)\pi_2^H - 0.40(\pm 0.14)\Sigma\alpha_2^H - 1.69(\pm 0.18)\Sigma\beta_2^0 - 0.05(\pm 0.24) \quad (10)$$

with a multiple correlation coefficient $\rho = 0.997$, standard error (S.E.) = 0.056, and Fischer *F*-statistic (F) = 148. Statistically the fit is good and the system constants make chemical sense (they show good agreement with values from larger data sets presented later). The model is adequate and in the absence of additional data there is little that can be done to improve the standard deviations in the system constants to refine the fit.

Vitha et al. [42,48] used headspace gas chromatography to determine the binding constant ($\log K_M$) for 22 solutes in the system sodium dodecyl sulfate–water, Table 3. Originally these authors published data for the first 20 solutes in Table 3 and then later added data for aniline and 2,6-dimethylaniline when they attempted to provide a model for solute binding.

Table 2
System constants, experimental data and cross-correlation matrix for the sodium dodecyl sulfate system studied by Chen et al. [25]

Solute	Descriptors					$\log K_X$
	V_X	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$	
Phenol	0.7751	0.805	0.89	0.60	0.31	1.04
Benzyl alcohol	0.9160	0.803	0.87	0.33	0.56	1.10
Aniline	0.8162	0.955	0.96	0.26	0.50	0.96
Toluene	0.8573	0.601	0.52	0	0.14	1.83
Ethylbenzene	0.9982	0.613	0.51	0	0.15	2.22
Naphthalene	1.0854	1.340	0.92	0	0.20	2.49
Benzaldehyde	0.8730	0.820	1.00	0	0.39	1.39
Nitrobenzene	0.8906	0.871	1.11	0	0.28	1.47
Chlorobenzene	0.8388	0.718	0.65	0	0.07	1.94
Acetophenone	1.0139	0.818	1.00	0	0.49	1.58
Phenylacetone	1.1548	0.748	0.90	0	0.66	1.55
Cross-correlation matrix (r^2)						
V_X	1.000					
R_2	0.067	1.000				
π_2^H	0.004	0.313	1.000			
$\Sigma\alpha_2^H$	0.107	0.213	0.023	1.000		
$\Sigma\beta_2^0$	0.097	0.005	0.342	0.059	1.000	

Table 3
System constants, experimental data and cross-correlation matrix for the sodium dodecyl sulfate system studied by Vitha et al. [42,48]

Solute	Descriptors					$\log K_M$
	V_X	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$	
Methanol	0.3082	0.278	0.44	0.43	0.47	0.0792
Ethanol	0.4491	0.246	0.42	0.37	0.48	0.3802
1-Propanol	0.5900	0.236	0.42	0.37	0.48	0.8808
1-Butanol	0.7309	0.224	0.42	0.37	0.48	1.2625
1-Pentanol	0.8718	0.219	0.42	0.37	0.48	1.8351
1-Hexanol	1.0127	0.210	0.42	0.37	0.48	2.2876
Benzene	0.7164	0.610	0.52	0	0.14	2.0137
Toluene	0.8573	0.601	0.52	0	0.14	2.4208
Ethylbenzene	0.9982	0.613	0.52	0	0.15	2.7782
Propylbenzene	1.1391	0.604	0.50	0	0.15	3.2079
Butylbenzene	1.2800	0.600	0.51	0	0.15	3.6278
Pentylbenzene	1.4209	0.594	0.51	0	0.15	3.9605
2-Butanone	0.6879	0.166	0.70	0	0.51	1.1206
2-Pentanone	0.8288	0.143	0.68	0	0.51	1.5599
2-Hexanone	0.9676	0.136	0.68	0	0.51	1.9791
2-Heptanone	1.1106	0.123	0.68	0	0.51	2.4378
2-Nonanone	1.3924	0.119	0.68	0	0.51	3.3345
1-Nitrobutane	0.8464	0.227	0.95	0	0.29	1.8096
1-Nitropentane	0.9873	0.212	0.95	0	0.29	2.2279
1-Nitrohexane	1.1282	0.203	0.95	0	0.29	2.6964
Aniline	0.8162	0.955	0.96	0.26	0.50	1.4099
2,6-Dimethylaniline	1.0980	0.972	0.89	0.20	0.46	2.1590
Cross-correlation matrix (r^2): *first 20 solutes*						
V_X	1.000					
R_2	0.046	1.000				
π_2^H	0.077	0.129	1.000			
$\Sigma\alpha_2^H$	0.372	0.177	0.391	1.000		
$\Sigma\beta_2^0$	0.133	0.818	0.002	0.252	1.000	
Cross-correlation matrix (r^2): *all 22 solutes*						
V_X	1.000					
R_2	0.033	1.000				
π_2^H	0.063	0.016	1.000			
$\Sigma\alpha_2^H$	0.329	0.000	0.168	1.000		
$\Sigma\beta_2^0$	0.120	0.206	0.006	0.280	1.000	

Using the first 20 solutes in Table 3 we obtained the following fit

$$\log K_M = 3.00(\pm 0.09)V_X - 0.63(1.12)R_2 - 0.97(0.64)\pi_2^H - 0.55(\pm 0.33)\Sigma\alpha_2^H - 2.29(\pm 1.17)\Sigma\beta_2^0 + 1.00(\pm 1.25) \quad (11)$$

with $\rho = 0.999$, S.E. = 0.06 and $F = 1017$. Statistically the fit is good but the large standard deviation in the r and b constants indicates a problem. Since the solute descriptors for hydrogen-bond acidity are clustered ($\Sigma\alpha_2^H$) this might be interpreted as the cause of the poor model. Inspection of the cross-correlation table indicates that R_2 and $\Sigma\beta_2^0$ are unintentionally correlated and the addition of the two hydrogen-bond acid solutes, aniline and 2,6-dimethylaniline, not only improves the estimate of the

a constant but removes the correlation between R_2 and $\Sigma\beta_2^0$. For all 22 solutes the fit is

$$\log K_M = 3.02(\pm 0.07)V_X - 0.58(0.09)\pi_2^H - 0.37(\pm 0.14)\Sigma\alpha_2^H - 1.65(\pm 0.12)\Sigma\beta_2^0 + 0.31(\pm 0.10) \quad (12)$$

with $\rho = 0.998$, S.E. = 0.071 and $F = 1035$. There is very little difference in the statistics of the fit between Eqs. (11) and (12) but Eq. (12) is clearly a better model from a chemical sense. Note that in Eq. (12) the *r* constant is not significant and forcing a fit of Eq. (11) with $r = 0$ would produce essentially the same model as Eq. (12), confirming that in this case cross-correlation was the reason that a poor model was obtained in Eq. (11). There is good general agreement in the contribution of intermolecular interactions to solubilization in sodium dodecyl sulfate micelles indicated by the models given by Eqs. (10) and (12) (*c* constants are not expected to agree because the dependent variables are different) given that Eq. (10) refers to a sodium borate buffer as the aqueous phase and Eq. (12) to water. It is an established fact that the size, shape and counterion binding of micelles is influenced by the presence of electrolyte in the aqueous phase, and consequently some changes in solvation characteristics are to be anticipated [18,43,49,50].

Adlard et al. [51] have provided retention factors by MEKC for 18 varied solutes using potassium deoxycholate (KDC) and potassium 3β-glucopyranosyl-5β-cholan-12α-hydroxy-24-oic acid (KGDC) surfactants in a 50 m*M* potassium borate buffer at pH 8 or 9 containing 40 m*M* of surfactant, Table 4. The main concern here (besides the small number of solutes) is that the values for the dependent variable are, in general, small and clustered (possibility that the retention factors contain a larger error than is

Table 4
System constants, experimental data and cross-correlation matrix for the potassium deoxycholate (KDC) and its glucopyranose derivative (KGDC) studied by Adlard et al. [51]

Solute	Descriptors					Experimental data (log *k*)		
	V_X	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$	KDC pH 8	KDC pH 9	KGDC pH 9
Benzyl alcohol	0.9160	0.803	0.87	0.33	0.56	−0.853	−0.853	−0.886
Phenol	0.7751	0.805	0.89	0.60	0.31	−0.677	−0.552	−0.602
Acetophenone	1.0139	0.818	1.01	0	0.49	−0.468	−0.455	−0.537
Nitrobenzene	0.8906	0.871	1.11	0	0.28	−0.481	−0.443	−0.060
Benzene	0.7164	0.610	0.52	0	0.14	−0.408	−0.376	−0.443
Anisole	0.9160	0.708	0.75	0	0.29	−0.301	−0.259	−0.337
Methyl 4-nitrobenzoate	1.2468	0.950	1.38	0	0.57	−0.244	−0.229	−0.237
2-Methylbenzonitrile	1.0120	0.780	1.06	0	0.31	−0.214	−0.193	−0.284
3-Nitrotoluene	1.0315	0.874	1.10	0	0.28	−0.022	0.004	0.692
Toluene	0.8573	0.601	0.52	0	0.14	0.134	0.164	0.079
2-Naphthol	1.1440	1.520	1.08	0.61	0.40	0.308	0.428	0.305
Bromobenzene	0.8914	0.882	0.73	0	0.09	0.417	0.438	0.369
Benzophenone	1.4810	1.447	1.50	0	0.50	0.614	0.669	0.520
1,4-Dimethylbenzene	0.9982	0.613	0.52	0	0.16	0.649	0.650	0.569
1-Nitronaphthalene	1.2596	1.600	1.51	0	0.29	0.728	0.625	1.142
4-Phenylphenol	1.3829	1.560	1.41	0.59	0.45	0.701	0.729	0.643
Naphthalene	1.0854	1.340	0.92	0	0.20	0.883	0.857	0.755
Butylbenzene	1.2800	0.600	0.51	0	0.15	1.502	1.298	1.227
Cross-correlation matrix (r^2)								
V_X	1.000							
R_2	0.460	1.000						
π_2^H	0.441	0.618	1.000					
$\Sigma\alpha_2^H$	0.003	0.144	0.036	1.000				
$\Sigma\beta_2^0$	0.197	0.154	0.469	0.126	1.000			

desirable and limitations in fitting the model by multiple linear regression), there are only four values for $\Sigma\alpha_2^H$ which are clustered, and three of the solutes with an $\Sigma\alpha_2^H$ value are phenols and possibly partially ionized at pH 9. Phenol could be expected to be about 13% dissociated and 2-naphthol about 31% dissociated at pH 9. The ionized form of the phenols is expected to have different solubility in the micelles to the neutral form and since both the micelles and ionized form of the phenols have the same charge, ionic repulsion may contribute to changes in retention as well. Common sense dictates that we have to be a little circumspect in our interpretation of the results from this data set. The models for potassium deoxycholate at pH 8 and 9 are set out below

$$\log k(\text{pH}=8) = 3.10(\pm0.17)V_X - 0.53(\pm0.16)R_2 - 0.92(\pm0.17)\pi_2^H - 2.50(\pm0.27)\Sigma\beta_2^0 - 1.97(\pm0.12) \quad (13)$$

$\rho = 0.991$, S.E. = 0.10 and $F = 139$.

$$\log k(\text{pH}=9) = 2.83(\pm0.15)V_X - 0.57(\pm0.14)R_2 - 0.85(\pm0.15)\pi_2^H - 2.40(\pm0.24)\Sigma\beta_2^0 - 1.82(\pm0.11) \quad (14)$$

$\rho = 0.993$, S.E. = 0.09 and $F = 160$.

The statistics are reasonable and the coefficients sensible so the models are useful for qualitative purposes. However, we cannot use Eq. (13) as a qualitative check on the influence of dissociation on the model at the higher pH, Eq. (14), because although we do not expect the solubility or adsorption of neutral molecules to be influenced by pH the properties of the micelles, which are acidic substances, cannot be assumed to be independent of pH. The glucopyranose derivative of potassium deoxycholate is a novel surfactant and we would like to ascertain the influence of derivatizing the C-5 hydroxyl group with the hydrophillic glucopyranose group on selectivity. The fit for the model obtained at pH 9 is given below

$$\log k = 2.61(\pm0.32)V_X - 0.50(\pm0.26)R_2 - 0.48(\pm0.31)\pi_2^H - 2.48(\pm0.37)\Sigma\beta_2^0 - 1.85(\pm0.25) \quad (15)$$

$\rho = 0.960$, S.E. = 0.19 and $F = 38$.

The fit for Eq. (15) is clearly not as good as Eq. (14). Inspection of predicted and experimental values of $\log k$ indicates that nitronaphthalene is a clear outlier as far as the model is concerned ($\log k$ experimental = 1.142 and predicted = 0.797). Comparing the average change in values for the solutes in Table 4 subjectively indicates that the experimental value for nitronaphthalene is out of line with the other solutes. Removing nitronaphthalene improves the fit, Eq. (16), and significantly changes the importance of dipole-type interactions in the model

$$\log k = 2.62(\pm0.26)V_X - 0.46(\pm0.22)R_2 - 0.69(\pm0.26)\pi_2^H - 2.21(\pm0.32)\Sigma\beta_2^0 - 1.74(\pm0.21) \quad (16)$$

$\rho = 0.970$, S.E. = 0.16 and $F = 48$.

In the new model the agreement between the predicted and experimental values of nitrobenzene ($\log k$ experimental = −0.061 and predicted = −0.388) is not good, with the difference for nitrobenzene being over two standard deviations. We could legitimately remove nitrobenzene as an outlier to give Eq. (17)

$$\log k = 2.78(\pm0.20)V_X - 0.60(\pm0.17)R_2 - 1.03(\pm0.22)\pi_2^H - 1.99(\pm0.25)\Sigma\beta_2^0 - 1.83(\pm0.16) \quad (17)$$

$\rho = 0.970$, S.E. = 0.12 and $F = 89$.

This model is about as good as we can achieve with the data available. Eqs. (15)–(17) illustrate one of the main problems with small data sets. There is very little protection from the presence of outliers; but the main problem with the data set in Table 4 is that there are too many solutes with low k values, which are likely to contain a disproportionately large experimental error. For the purpose of constructing a model a wider range of retention properties is preferable and the preliminary model given by Eq. (17) could be used as a first round estimate to identify solutes with appropriate retention charac-

teristics. If we return to our original question, what is the influence of replacing the C-5 hydroxyl group by a glucopyranose derivative on the selectivity of potassium deoxycholate, we can gain some insight by comparison of the models given by Eqs. (14) and (17). With reasonable confidence we can state that the introduction of the glucopyranose derivative has little influence on selectivity with the difference in the *s* and *b* system constants being barely significant at the 95% confidence level for the two models. A somewhat surprising result, but an indication that the glucopyranose group must be somewhat removed from the location at which solute sorption occurs. We can gain some confidence in the above predictions by noting that the system constants for potassium deoxycholate are very similar to those for sodium deoxycholate [52], obtained under more favorable conditions, and presented in the Section 5.

Large data sets of carefully chosen varied solutes are preferred for determining the selectivity of micellar phases in MEKC. It is inevitable that small data sets will be used from time to time since these are the type most commonly found in the chemical literature. The purpose of this section was to illustrate some of the problems and pitfalls that can be encountered in their interpretation if adequate common sense precautions are not taken. Often a good statistical fit will be obtained, but the system constants will not be sensible, and it is important that the difference between a fit and a model is appreciated. The former may be adequate to explain retention changes within the data collection but cannot be used to interpret the results in terms of fundamental interactions, and will not necessarily accurately predict retention of solutes not contained in the data collection used to generate the fit. We will now consider some more secure models for the purpose of characterizing selectivity of surfactant systems in MEKC.

5. Compilation of system constants from large data sets by MEKC

The most studied surfactant in MEKC is sodium dodecyl sulfate. In Table 5 are collected the system constants and experimental conditions for the data sets reported by Poole and Poole [52], Muijselaar et al. [30] (including results for tris[hydroxymethyl]-aminomethane dodecyl sulfate and sodium dodecyl sulfonate), Herbert and Dorsey [53] and Yang et al. [27] together with the results calculated in Section 4 for Chen et al. [25]. Even though the experimental conditions vary between studies there is good general agreement among the models. The characteristics which favor sorption by the micelles (system constants are positive) are their lower cohesion compared to the support electrolyte (*m* constant) and favorable lone pair–lone pair electron attraction (*r* constant). All polar interactions of a dipole-type (*s* constant) and solute hydrogen-bond acidity (*a* constant) weakly favor, and solute hydrogen-bond basicity (*b* constant) strongly favor, solubility in the support electrolyte. Given the narrow range of system constants in Table 5, the retention of neutral solutes by sodium dodecyl sulfate micelles cannot be too sensitive to changes in pH (7.0 to 8.5) and buffer type and concentration (sodium phosphate and sodium borate from 20 to 160 m*M* and Tris 20 m*M*). The concentration of sodium dodecyl sulfate (20 to 150 m*M*) does not have a large effect on selectivity but influences retention through changes in the phase ratio (seen, in part, in the model constant, *c* term). Replacing sodium by the tris(hydroxymethyl)aminomethane cation or the sulfate group by sulfonate has little influence on selectivity.

The bile salt surfactants are probably the second most widely used group of surfactants in MEKC after sodium dodecyl sulfate. The system constants for sodium cholate under various experimental conditions and sodium deoxycholate, sodium taurocholate, and sodium taurodeoxycholate are summarized in Table 6 [27,52]. All the bile salts are stronger hydrogen-bond bases than sodium dodecyl sulfate, but solute hydrogen-bond acidity does not contribute significantly to retention, since the *a* constant is zero or very small for the bile salts. The solvated bile salt micelles have a similar hydrogen-bond basicity as the buffer, while the solvated sodium dodecyl sulfate micelles are less competitive with the buffer resulting in a preference for hydrogen-bond acid solutes to solubilize in the buffer. The bile salts are all weaker hydrogen-bond acids than sodium dodecyl sulfate and compete even less effectively with the buffer for the sorption of hydrogen-bond acid solutes. The bile salts are slightly more cohesive than

Table 5
Examples of model fits for sodium dodecyl sulfate and related surfactants in MEKC

System constants						Statistics	Conditions	Reference
m	*r*	*s*	*a*	*b*	*c*			for data
2.99	0.46	−0.44	−0.30	−1.88	−1.82	$\rho=0.994$	Surfactant=50 m*M*, buffer sodium phosphate–sodium	[52]
(0.07)	(0.05)	(0.05)	(0.05)	(0.08)	(0.07)	S.E.=0.07	borate 10+10 m*M*, pH=8, temp.=25°C, $n=40$	
						$F=569$		
2.62	0.56	−0.67	−0.31	−1.57	−1.48	$\rho=0.996$	Surfactant=50 m*M*, buffer sodium phosphate 20 m*M*	[30]
(0.07)	(0.09)	(0.07)	(0.07)	(0.08)	(0.07)	S.E.=0.07	pH=7, temp.=25°C, $n=26$	
						$F=517$		
2.91	0.31	−0.24	−0.44	−1.87	−1.85	$\rho=0.994$	Surfactant=50 m*M*, buffer sodium phosphate–sodium	[53]
(0.17)	(0.08)	(0.08)	(0.08)	(0.15)	(0.15)	S.E.=0.11	borate 60+100 m*M*, pH=7, temp.=30°C, $n=32$	
						$F=397$		
2.83	0.47	−0.44	−0.15	−1.71	−2.17	$\rho=0.991$	Surfactant=20 m*M*, buffer sodium phosphate 50 m*M*,	[27]
(0.09)	(0.06)	(0.07)	(0.04)	(0.08)	(0.08)	S.E.=0.07	pH=7, temp.=25°C, $n=59$	
						$F=574$		
2.81	0.46	−0.48	−0.16	−1.71	−1.78	$\rho=0.991$	Surfactant=40 m*M*, other conditions as above	[27]
(0.09)	(0.06)	(0.07)	(0.04)	(0.08)	(0.08)	S.E.=0.07		
						$F=565$		
2.48	0.50	−0.53	−0.40	−1.69	−0.05	$\rho=0.997$	Surfactant=30 to 150 m*M* ($\log K_x$), buffer sodium	[25]
(0.26)	(0.15)	(0.17)	(0.14)	(0.18)	(0.24)	S.E.=0.06	borate 25 m*M*, pH 8.5, temp.=25°C, $n=11$	
						$F=148$		
Tris(hydroxymethyl)aminomethane dodecyl sulfate								
2.56	0.57	−0.66	−0.33	−1.56	−1.43	$\rho=0.997$	Surfactant 50 m*M*, buffer Tris 20 m*M*, pH=7,	[30]
(0.07)	(0.09)	(0.07)	(0.07)	(0.07)	(0.06)	S.E.=0.06	temp.=25°C, $n=24$	
						$F=532$		
Sodium dodecyl sulfonate								
2.51	0.51	−0.70	−0.14	−1.51	−1.47	$\rho=0.997$	Surfactant 50 m*M*, buffer Tris 20 m*M*, pH=7,	[30]
(0.07)	(0.08)	(0.07)	(0.06)	(0.07)	(0.06)	S.E.=0.06	temp.=40°C, $n=24$	
						$F=557$		

sodium dodecyl sulfate and are expected to exhibit lower retention of solutes with weak polar interactions. The capacity for dipole-type interactions and lone pair–lone pair electron interactions of the bile salts and sodium dodecyl sulfate are similar. As a group the bile salts do show a modest range of selectivity differences, largely in their capacity as hydrogen-bond acids, but these differences will only amount to small retention changes and a more radical solution is needed for selectivity optimization.

In Table 7 are summarized the system constants for some miscellaneous surfactants and a microemulsion [27,29,52,54]. Lithium perfluorooctanesulfonate has very different sorption properties to the bile salts and sodium dodecyl sulfate. It is a much stronger hydrogen-bond acid and weaker hydrogen-bond base, is more cohesive, and is significatly more dipolar and/or polarizable. The negative *r* constant is characteristic of fluorinated compounds and is a result of the tighter binding of electron pairs due to the inductive effect of fluorine compared to a normal hydrocarbon. N-Dodecanoyl-N-methyltaurine and the two cationic surfactants, tetradecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide are distinguished from the other surfactants by their strong hydrogen-bond basicity (positive *a* constant) and weak hydrogen-bond acidity with respect to the buffer.

The selectivity of the various micelles for polar interactions are easiest to compare by normalizing the system constants for polar interactions by division with the *m* constant, the ratio representing the capacity of the micelles for polar interactions independent of solute size. For simplicity we have averaged the multiple values for the system constants for sodium dodecyl sulfate (Table 5) and for sodium

Table 6
Examples of model fits for bile salt and surfactants in MEKC

System constants						Statistics	Conditions
m	*r*	*s*	*a*	*b*	*c*		
Sodium cholate							
2.59	0.65	−0.47	0	−2.27	−2.11	$\rho=0.985$	Surfactant=50 m*M*, buffer sodium
(0.13)	(0.09)	(0.10)		(0.13)	(0.13)	S.E.=0.11	phosphate–sodium borate 10+10 *M*,
						$F=275$	pH=8, temp.=25°C, $n=40$
2.45	0.63	−0.47	0	−2.29	−1.71	$\rho=0.983$	Surfactant=75 m*M*, other
(0.12)	(0.08)	(0.09)		(0.13)	(0.12)	S.E.=0.11	conditions as above
						$F=241$	
2.39	0.48	−0.46	0	−2.14	−1.34	$\rho=0.986$	Surfactant=125 m*M*, other
(0.10)	(0.07)	(0.08)		(0.12)	(0.10)	S.E.=0.10	conditions as above
						$F=281$	
2.65	0.56	−0.74	0.15	−2.49	−1.69	$\rho=0.985$	Surfactant=60 m*M*, buffer sodium
(0.12)	(0.08)	(0.10)	(0.06)	(0.11)	(0.11)	S.E.=0.10	phosphate 50 m*M*, pH=7, temp. 25°C
						$F=337$	$n=59$
2.66	0.50	−0.76	0.12	−2.52	−1.48	$\rho=0.986$	Surfactant=80 m*M*, other conditions as above
(0.13)	(0.08)	(0.10)	(0.06)	(0.11)	(0.11)	S.E.=0.10	$n=59$
						$F=294$	
Sodium taurocholate							
2.43	0.60	−0.34	0	−2.06	−2.10	$\rho=0.989$	Surfactant=50 m*M*, buffer sodium
(0.09)	(0.07)	(0.07)		(0.10)	(0.09)	S.E.=0.09	phosphase–sodium borate 10+10 m*M*,
						$F=377$	pH=8, temp.=25°C, $n=40$
Sodium deoxycholate							
2.67	0.66	−0.47	0	−2.47	−1.69	$\rho=0.986$	Surfactant=75 m*M*, other
(0.11)	(0.08)	(0.09)		(0.13)	(0.12)	S.E.=0.11	conditions as above
						$F=286$	
Sodium taurodeoxycholate							
2.62	0.67	−0.45	0	−2.17	−1.99	$\rho=0.991$	Surfactant=50 m*M*, other
(0.09)	(0.06)	(0.07)		(0.10)	(0.09)	S.E.=0.09	conditions as above
						$F=430$	

cholate (Table 6), while recognizing that some difference in the system constants resulting from variation in the experimental conditions is extant in the averaged data. The results are summarized in Table 8. Method development usually begins with sodium dodecyl sulfate because of its favorable kinetic and chromatographic properties. Other surfactants should be selected based on their complementary properties to sodium dodecyl sulfate. We can immediately deselect sodium dodecyl sulfonate and tris(hydroxymethyl)aminomethane dodecyl sulfate as being too similar to sodium dodecyl sulfate to produce significant changes in selectivity. The tris(hydroxymethyl)aminomethane dodecyl sulfonate salt is an interesting case since some of the cations must be bound to the surface layer of the micelle and might have been expected to have a larger influence on the sorption properties of the micelle than those observed. Sodium cholate selects itself as an example of a bile salt which differs from sodium dodecyl sulfate mainly in its capacity for hydrogen-bond interactions (stronger base/weaker acid). Having selected sodium cholate the other bile salts deselect themselves except for fine tuning of a nearly acceptable separation. Also, the microemulsion has selectivity similar to sodium cholate (bile salts in general) and deselects itself. Lithium perfluorooctanesulfonate has little in common with the other surfactants and selects itself. It has different selectivity for lone pair–lone pair electron interactions (the only nega-

Table 7
Miscellaneous surfactants characterized by MEKC

System constants						Statistics	Conditions
m	*r*	*s*	*a*	*b*	*c*		
N-Dodeconyl-N-methyltaurine							
3.07	0.72	−0.50	0.22	−2.58	−2.01	$\rho=0.992$	Surfactant=50 m*M*, buffer sodium
(0.09)	(0.06)	(0.07)	(0.06)	(0.10)	(0.09)	S.E.=0.11	phosphate–sodium borate 10+10 m*M*,
						$F=338$	pH=8, temp.=25°C, $n=39$
Lithium perfluorooctanesulfonate							
2.30	−0.52	0.34	−0.82	−0.53	−2.01	$\rho=0.977$	Surfactant=40 m*M*, buffer sodium
(0.12)	(0.08)	(0.09)	(0.06)	(0.10)	(0.11)	S.E.=0.10	phosphate 50 m*M*, pH=7, temp.=
						$F=218$	25°C, $n=59$
Tetradecyltrimethylammonium bromide							
2.82	0.36	−0.29	0.90	−2.67	−2.10	$\rho=0.986$	Surfactant=10 m*M*, other
(0.11)	(0.07)	(0.09)	(0.05)	(0.09)	(0.10)	S.E.=0.09	conditions as above
						$F=380$	
Hexadecyltrimethylammonium bromide							
3.40	0.61	−0.55	0.58	−3.08	−1.67	$\rho=0.993$	Surfactant=50 m*M*, sodium phosphate–sodium
(0.10)	0.06)	(0.07)	(0.06)	(0.10)	(0.11)	S.E.=0.08	borate buffer 10+10 m*M*,
						$F=436$	pH=7, temp.=25°C, $n=36$
Emulsion (1.4% wt. sodium dodecyl sulfate, 6.49% wt. butan-1-ol and 0.82% wt. heptane)							
3.05	0.28	−0.69	−0.06	−2.81	−1.13	$\rho=0.994$	Buffer sodium phosphate–sodium borate
(0.08)	0.06)	(0.07)	(0.05)	(0.09)	(0.07)	S.E.=0.09	500+100 m*M*, pH=7, temp.=
						$F=791$	25°C, $n=53$

tive *r* constant), it is the most dipolar (the only positive *s* constant) and is the strongest hydrogen-bond acid and weakest hydrogen-bond base of the surfactants considered. N-Dodecanoyl-N-methyltaurine and hexadecyltrimethylammonium bromide are close enough in properties that we do not need both surfactants and would probably select the anionic surfactant for convenience, but tetradecyltrimethylammonium bromide is a significantly stronger hydrogen-bond base than the other two

Table 8
Ratio of system constants for surfactant systems studied by MEKC

Surfactant	*r*/*m*	*s*/*m*	*a*/*m*	*b*/*m*
Sodium dodecyl sulfate	0.15	−0.15	−0.10	−0.63
Tris(hydroxymethyl)aminomethane dodecyl sulfate	0.21	−0.26	−0.13	−0.62
Sodium dodecyl sulfonate	0.18	−0.26	−0.06	−0.62
Sodium cholate	0.22	−0.20	0	−0.94
Sodium taurocholate	0.25	−0.14	0	−0.85
Sodium deoxycholate	0.25	−0.18	0	−0.93
Sodium taurodeoxycholate	0.26	−0.17	0	−0.83
N-Dodecanoyl-N-methyltaurine	0.23	−0.16	0.07	−0.84
Lithium perfluorooctanesulfonate	−0.23	0.15	−0.36	−0.23
Tetradecyltrimethylammonium bromide	0.13	−0.10	0.32	−0.95
Hexadecyltrimethylammonium bromide	0.18	−0.16	0.17	−0.91
Emulsion (see Table 7 for details)	0.09	−0.23	−0.02	−0.92

surfactants and has to be retained for selectivity optimization. Thus from the information currently available a working list of surfactants for selectivity optimization in MEKC would include sodium dodecyl sulfate, sodium cholate, lithium perfluorooctanesulfonate, N-dodecanoyl-N-methyltaurine and tetradecyltrimethylammonium bromide.

There is only limited information concerning the use of mixed surfactant micelles and organic solvent additives in MEKC and their influence on system constants [18,30,55,56]. Nonionic surfactants as a component of mixed surfactant micelles allow changes in selectivity, phase ratio and separation time without affecting the operating current and usually without degrading efficiency in MEKC. Systematic studies of the influence of the mole ratio of Brij 35, a neutral polyoxyethylene[23]dodecyl ether, on the selectivity of mixed surfactant micelles containing sodium dodecyl sulfate [30,56] and sodium N-dodecanoyl-N-methyltaurine [55] produced similar trends. As shown in Fig. 1, the addition of Brij 35 to sodium dodecyl sulfate resulted in only small changes in the *m*, *r* and *s* system constants of the solvation parameter model; a slight increase in the hydrogen-bond basicity of the mixed surfactant micelle (*a* constant); and at low concentration a significant change in the hydrogen-bond acidity of the mixed surfactant micelle (*b* constant). The addition of organic solvent to a surfactant system produces changes in selectivity reminiscent of the results observed in bonded-phase, reversed-phase liquid chromatography, except that the range of modifier concentration is restricted to predominantly aqueous solutions because of instability of the micelles at high modifier concentrations. The system constants, as a function of the volume of organic solvent, change in a smooth and regular fashion permitting the prediction of retention in a manner useful for methods development [55]. Our understanding of selectivity with respect to the properties of mixed surfactant micelles and the use of organic solvent modifiers is weak, but these preliminary studies indicate that the solvation parameter model can be used to characterize these systems which promise a flexible approach to tailoring selectivity properties for a particular separation, at least over modest ranges of system constant changes.

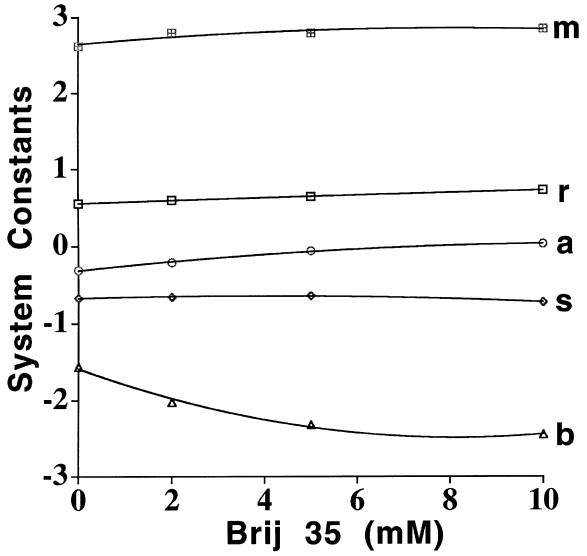

Fig. 1. Plot of the variation of the system constants with composition of a mixed surfactant buffer containing 50 m*M* sodium dodecyl sulfate and 0 to 10 m*M* Brij 35. The system constants were calculated from experimental data given in Ref. [30].

6. Recommendation for generic experimental conditions to determine selectivity of anionic micelles

Micelles are solvents with properties that depend to some extent on their external environment, particularly the ionic strength, choice of counterion, pH and temperature of the supporting buffer. For the purpose of characterizing their sorption characteristics in MEKC it would be useful to have a generic set of measurement conditions suitable for standardization, then other conditions could be introduced to establish the influence of experimental parameters on the observed chromatographic selectivity using the solvation parameter model. As a first step to achieving this goal we would like to suggest a generic set of conditions for determining the selectivity of anionic surfactants in MEKC. Our experience with cationic micelles is too shallow to indicate either a separate set of conditions for their characterization or to confirm whether a single set of conditions can be employed for both types of surfactants.

The electroosmotic flow is dependent largely on the buffer composition and pH. A pH of 8 provides a reasonable electroosmotic velocity with fused silica capillary columns. A 20 m*M* sodium phosphate buffer should provide the necessary pH control with sufficient buffer capacity to avoid retention changes due to electrolysis of the buffer at normal operating voltages. Its concentration is low enough that Joule heating will not be a problem for typical capillaries. Many anionic surfactants are available as the sodium salt and it is adventitious to maintain the same counterion for the buffer and the surfactant to avoid a further factor that might influence the sorption characteristics of the micelles. A temperature of 25°C is reasonable for measurements unless solubility or solution viscosity problems indicate that another temperature should be used. The surfactant concentration should be greater than the critical micelle concentration but generally less than 200 m*M*. The critical micelle concentration is generally less for ionic solutions than the values for water found in common reference books. In the region of the critical micelle concentration, variations in micelle properties can be significant, so that whenever possible, a surfactant concentration about five times the critical micelle concentration should be used. Very high surfactant concentrations, however, are undesirable because they result in excessive current and high solution viscosities, and because micelle properties are likely to be influenced by changes in the shape and aggregation number of the micelles. Retention reproducibility needs to be assured by using an effective cleaning and conditioning step in the measurement sequence. Sequential rinsing with sodium hydroxide solution, buffer without surfactant, and some times an organic solvent is needed; these conditions, concentration, time, etc., are usually established by trial and error and are likely to be different for different surfactants. Once a capillary has been employed to make a series of measurements with one surfactant it can be difficult to regenerate it for use with a different surfactant. It is better to start each series of measurements for a new surfactant with a fresh capillary column. Normally injected analyte quantities in MEKC are small by comparison to the typical concentration of micelles in the buffer. Large sample volumes or high sample concentrations should be avoided as they may influence retention through disruption of the electrophoretic conditions in the column or exceed the range over which the sorption properties of the micelle can be considered independent of solute concentration. It seems that because of poor kinetic properties or adsorption at the column wall that there are always a few solutes in any solute collection, and not necessarily the same solutes with different surfactants, that have poor peak shapes, the retention of which cannot be determined with sufficient accuracy by the peak maxima method to retain for modeling.

7. Conclusions

Only a small fraction of known surfactants, or even surfactant types, have been evaluated for use in MEKC. The characterization of surfactants by comparison of peak positions between chromatograms is unsatisfactory because it does not lead to a fundamental understanding of surfactant behavior and is subject to misinterpretation, since individual compounds with a dominant single polar intermolecular interaction are not available. In addition, a comparison of results between single surfactants will often demonstrate selectivity differences which would be less encouraging if compared against a number of surfactants simultaneously. Given a large enough group of surfactants most will be deemed to have similar selectivity while the need is to identify a small group of surfactants that can represent the general sorption properties of a larger group as either single or blended surfactant mixtures. We wish to avoid the problem that arose in the early days of gas chromatography when a large number of stationary phases were introduced with individual claims of unique or different separation properties later to be disproved when reliable methods of characterization came into being. The solvation parameter model provides a tool to understand the fundamental basis of solute–micelle interactions and to characterize surfactants under conditions germane to their use in MEKC. The experimental and mathematical conditions for the use of this model to characterize surfactant selectivity were presented along with applications of their use. A generic set of measurement conditions for anionic surfactants are suggested to standardize the conditions for the measurement of

selectivity in MEKC and to minimize the contribution from variations in selectivity that result from the influence of environmental factors on the sorption properties of micelles.

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