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Review

Interphase model for retention and selectivity in micellar electrokinetic chromatography

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Abstract

The fundamental properties of micelles are reviewed to arrive at a working definition of a micellar solvent for micellar electrokinetic chromatography (MEKC). Emphasis is placed on the dynamic nature of micelles, the influence of the external electrolyte environment on micelle properties, and factors that influence solubility of neutral solutes in micelles. The solvation parameter model is used to characterize the capacity of common surfactants for defined intermolecular interactions as a basis for understanding selectivity in MEKC. The need for additional surfactants and their required solvation properties to provide a wider selectivity range for methods development is identified. As an approach to understanding the mechanism of retention in MEKC an interphase model is proposed. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Retention models; Method development; Buffer composition; Reviews

Contents

1. Introduction

Micellar electrokinetic chromatography (MEKC) is one of a family of capillary electrophoretic techniques that includes capillary zone electrophoresis, capillary gel electrophoresis, capillary isotachophoresis, and capillary electrochromatography [1–9]. Several of these techniques, including MEKC, are now commonplace laboratory tools supported by a buoyant applications literature. The main feature they share in common is that they are all microcolumn techniques that employ an electric field to

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generate movement of ions and neutral molecules by electrophoresis and electroosmosis. The distinguishing feature of MEKC is the incorporation of a charged surfactant, above its critical micelle concentration in the separation buffer, such that when an electric field is applied along the capillary a separation of neutral molecules according to their distribution between the bulk electrolyte and a pseudostationary phase (micellar phase), is possible. In the above system ionic solutes are separated by a combination of distribution between phases and electrophoretic migration in the running buffer. Unlike other chromatographic systems, the micellar phase is termed a pseudostationary phase rather than a stationary phase, since it migrates in the supporting buffer with a direction and/or velocity that is different to that of the mobile phase.

High separation efficiencies can be achieved in MEKC (ca.>200 000 theoretical plates/meter) but the peak capacity is restricted by the migration window established by the migration time of an unretained solute (electroosmotic velocity of the mobile phase) and the effective migration velocity of the micelles in the direction of general flow of the bulk electrolyte. Still, within this operating window, complex mixtures can be separated by a combination of chromatography and electrophoresis, as demonstrated by the separation of aromatic compounds in Fig. 1. The high kinetic efficiency and the possibility of separating both neutral and ionized solutes in the same system are considerable attractions of MEKC, and this combined with the flexibility of adjusting selectivity by adding different complexing agents (e.g., cyclodextrins, urea, chiral additives, etc.), different surfactants, or organic solvents to the separation buffer, has considerably extended the general scope of the technique. The use of cyclodextrins in achieving the separation of isomers, enantiomers, and other difficult to separate compounds has to be highlighted as one of the major successes of MEKC. For example, the addition of γ-cyclodextrin to a buffer containing sodium dodecyl sulfate permitted the separation of 10 pharmaceutically important estrogens that were largely unresolved in the absence of the cyclodextrin (Fig. 2) [10]. On the other hand, the range of selectivity variation brought about by using different common surfactants or addition of organic solvents, appears

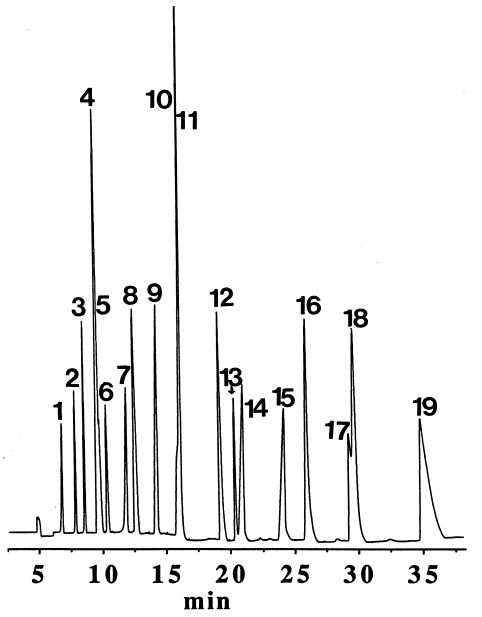

Fig. 1. Separation of aromatic compounds by MEKC. Separation buffer contains 10 m*M* each of sodium phosphate and sodium borate (pH 8) and 50 m*M* sodium dodecyl sulfate. The fused-silica separation capillary was 80.5 cm (effective length 72 cm)×50 μm I.D.; temperature, 35°C; and field strength, 30 kV. Compounds are: 1, benzenesulfonamide; 2, N-methylbenzamide; 3, acetanilide; 4, 2-phenylethanol; 5, 4-nitroaniline; 6, 3-cresol; 7, anisole; 8, methyl 3-hydroxybenzoate; 9, toluene; 10, chlorobenzene; 11, 3-nitotoluene; 12, 4-phenylphenol; 13, 2-naphthol; 14, ethylbenzene; 15, 1,4-dichlorobenzene; 16, naphthalene; 17, *n*-propylbenzoate; 18, 4-phenylphenol; and 19, *n*-butylbenzoate.

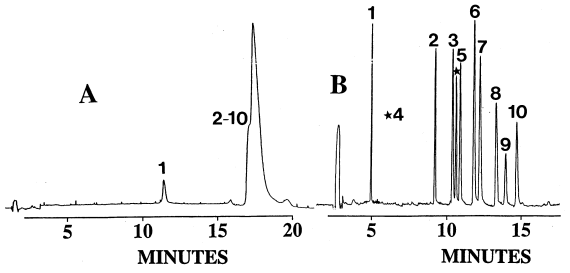

Fig. 2. Separation of pharmaceutically important estrogens by MEKC using (A) 10 m*M* each sodium phosphate and sodium borate buffer (pH 8) containing 50 m*M* sodium dodecyl sulfate and (B) the same system as (A) containing in addition 20 m*M* γ-cyclodextrin. The fused-silica separation capillary was 48.5 cm (effective length 40 cm)×50 μm I.D.; temperature, 25°C; and field strength, 20 kV. Compounds are: 1, estriol; 2, 17β-estradiol; 3, 17α-estradiol; 4, 17β-dihydroequilenin; 5, 17β-dihydroequilin; 6, 17α-dihydroequilenin; 7, 17α-dihydroequilin; 8, estrone; 9, equilenin; and 10, equilin.

not to be as powerful as is desirable. The theoretical and practical reasons for this observation will be outlined in this article.

Although many acceptable separations have been published using MEKC, the general approach to methods development is largely based on trial and error experiments assisted by some useful formal general observations [4,11–15]. A successful separation depends mainly on the choice of 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; and choice of additive (organic solvent, complexing agent, etc.), and its concentration. Normally, the experimental conditions are set to establish an acceptable separation time and migration window, under conditions where the efficiency is not compromised, and the outcome of the experiment controlled by selectivity optimization. Frequently the range of possible values for the experimental parameters are restricted by their interactions and needs of the experiment, simplifying method development at the expense of flexibility. It is generally agreed that the choice of surfactant is the most important consideration for optimizing selectivity. Formal models of retention in MEKC, the fundamental basis of any selectivity optimization approach, are few (discussed later) and rarely underpinned by connection to basic thermodynamic principles. Such models are required for a structure-led approach to computer-aided methods development and for a detailed understanding of solute–micelle interactions under different experimental conditions.

2. Micelles as solvents

Surfactants are long-chain hydrocarbon compounds with polar head groups. A general property of surfactants is the formation of micelles (molecular aggregates) when their concentration exceeds a threshold value, called the critical micelle concentration (cmc) [16–22]. The driving force for micelle formation in aqueous solution is the favorable free energy change accompanying segregation of the hydrocarbon chains from water, by packing the hydrocarbon chains into a central core surrounded by the polar head groups, thus minimizing the distortion of the solvent structure. This favorable free energy change is opposed by the electrostatic repulsion between head groups in ionic micelles and the steric repulsion of hydrated head groups in the case of nonionic micelles. Whether micellization occurs in a particular case, at what concentration, the aggregation number, the size, and the shape of the micelle depends on the balance between the factors promoting micellization and those opposing it.

The interior portion of a micelle is hydrocarbon-like and somewhat fluid, consisting of intertwined, randomly orientated hydrocarbon groups, although the intrinsic viscosity of the micellar core may be an order of magnitude greater than that of a hydrocarbon with a similar chain length. The surface of the micelle consists of the polar head groups, bound counterions, and associated water molecules. The net charge of ionic micelles is less than the aggregation number, indicating that a large fraction of the counterions remain associated with the micelle; these counterions form the Stern layer at the micellar surface. The Stern layer constitutes the inner part of the electrical double layer surrounding the micelle. The outermost boundary of the Stern layer corresponds to the hydrodynamic shear surface of the micelle and the core and the Stern layer constitute the kinetic micelle characterized by a surface potential (the electrophoretic zeta potential). The outer, more diffuse layer of the micelle, containing the remaining counterions to maintain electrical neutrality, is termed the Gouy-Chapman layer.

In aqueous solution, the micelles of many common ionic surfactants, are spherical at concentrations ranging from the cmc to at least 10 times the cmc. At higher concentrations, or in the presence of electrolytes or organic additives, spherical micelles convert to ellipsoidal (e.g., globular, dumbbell, etc.), rod-like, or other nonspherical forms, in which the surfactant head groups pack closer together than at low concentrations, and in the absence of added electrolyte. Normally spherical and ellipsoidal micelles have low size dispersity characterized by a narrow range of aggregation numbers. A different realm of micellization behavior is observed, however, when micelles grow larger in size and rod-like

micelles are generated. These aggregates can be visualized as having a cylindrical middle part with two spherical endcaps, and are often characterized by a broad distribution of aggregation numbers.

The major factors that affect the value of the cmc and the size of ionic micelles are the nature of the polar head groups and associated counterions, the length and structure of the hydrocarbon chain, the concentration of added electrolyte, and temperature [17,21–25]. For solutions of ionic surfactants, the micelle shape and size may show abrupt changes when the concentration increases to a value much larger than the cmc, or when the concentration of added electrolyte has reached a threshold value. Above the threshold value for added electrolyte, rod-like micelles form because the presence of electrolyte ions near the polar head groups of the surfactant molecules decreases the repulsion force between the head groups. A reduction in the repulsion makes it possible for the surfactant molecules to approach each other more closely and form larger aggregates, which requires much more space for the hydrocarbon chains. Because a micelle has a small volume, it must change into a rod-like micelle to increase the volume-to-surface ratio. For sodium dodecyl sulfate (SDS), spherical micelles are formed above the cmc (8 m*M*) in water; at about 70 m*M* these change to an ellipsoidal form; addition of sodium chloride causes the spherical or ellipsoidal SDS micelles to change to rod-like micelles (the salt threshold value is roughly 70 m*M* for SDS concentrations 20–40 and 50 m*M* at 100 m*M* SDS) [24]. The aggregation number for spherical micelles at the cmc in water is about 60, but in the presence of added salt, this increases to about 120 in 300 m*M* sodium chloride solution (ellipsoidal micelles formed) [16,23]. Thus, either the addition of electrolyte, or increasing the concentration of an ionic surfactant, will increase the ionic strength, partially screening repulsive interactions between the ionic head groups, and causes the more densely packed nonspherical micellar forms to become thermodynamically more stable than spheres. The identity of the counterion bound to the micelle also affects the cmc, with those ions that bind most strongly causing a decrease in the cmc. Common anions and cations can be ranked according to their binding strength to ionic micelles [17]. Short chain alcohols and some additives, such as urea, are known to increase the cmc of surfactants through their influence on the structure of the solvent [26–28]. They are generally not incorporated into the structure of micelles, unlike long-chain alcohols, which tend to decrease the cmc with increasing concentration through their solubility in the micelles and by reduction of the electrostatic repulsion between ionic head groups. At relatively low organic solvent concentration ($<$25% v/v) aggregation of surfactant monomers in aqueous solution may be totally inhibited.

A characteristic property of micelles is their capacity to enhance the solubility of sparingly soluble organic compounds in water [17,21,29]. This solubilization is a consequence of the presence of hydrophobic domains in the surfactant aggregates which act as compatible microenvironments for the location of hydrophobic solutes. Micellar solubilization is of importance in many industrial processes, such as detergency, emulsion polymerization, oil recovery, etc., and of relevance to the use of micellar phases in chromatography. A significant body of work is based on the determination or application of systems in the region of the maximum additive concentration, representing saturation of the micellar phase at equilibrium with a saturated aqueous phase, conditions unlikely to be germane to chromatographic experiments, and therefore will not be discussed at length here.

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 as a chemical species. It is this dichotomy that makes an exact theory of solubilization by micellar phases difficult. The primary theoretical approaches to the problem are based on either a pseudophase model, mass action model, multiple equilibrium model, or the application of small system thermodynamics [20,21,30–34]. Technically, bulk thermodynamics should not apply to solute partitioning into small aggregates, since these solvents are interfacial phases with large surface-to-volume ratios. In contrast to bulk phases, whose properties are invariant with position, the properties of small aggregates are expected to vary with distance from the interface [32]. The lattice model of solute partitioning into micelles concludes that virtually all types of solutes should favor the interface

over the interior of a spherical micelle, while for cylindrical micelles, the internal distribution of solutes favors the core, except for non-ideal solutes which have a greater affinity for the interface. In the water–sodium dodecyl sulfate system, Vitha et al. [33] concluded that both polar and nonpolar solutes are solubilized at the interface in preference to the hydrocarbon core of the micelle at low solute concentrations. Mukerjee and Ko [31] formulated a two-state model for micellar solubilization, in which it is assumed that a solute may exist in a dissolved state in the micellar core, and also in an adsorbed state at the micelle–water interface. In the dissolved state, the solute is subjected to the Laplace pressure effect resulting from the curvature of the micelle–water interface, and is used to explain why the solubility of hydrocarbons in a micelle is lower than in an equivalent hydrocarbon solvent. The interfacial activity of polar molecules tends to make the adsorbed state of much greater importance than the dissolved state. It is likely that the interaction of solute polar groups, containing dipole and hydrogen-bond sites, at different micellar interfaces, involves significant contributions from interactions with the polar head groups of anionic, cationic and zwitterionic micelles. For the vast majority of solutes, Mukerjee and Ko hypothesise that the adsorbed state is primarily responsible for micellar solubilization, with increasing concentration of solute favoring a redistribution to the dissolved state. This simple two-site model can rationalize, at least qualitatively, the results of most solubilization studies. Nonetheless, it should be emphasized that the model is at best an oversimplification, which divides a 'continuum' of environments into two extreme types of sites [29].

Aamodt et al. [20] proposed that the micelle can be divided into two regions; the core region, where only solute molecules are allowed, and the palisade layer, where the surfactant molecules are anchored with their head groups at the interface pointing towards water (Fig. 3) [34]. The size of the micelle is not limited in this case (although the model assumes a spherical geometry which will not always be true), since the core can be expanded to accommodate more solute, only the radius of the palisade layer is restricted to the length of a surfactant molecule. The distribution of the surfactant molecules between the aqueous solution and the palisade layer of the micelle as well as the solute distribution between the micellar core and the palisade layer may be obtained from the condition that the chemical potential for a component should be equal in all regions of a system, when the system is in equilibrium. If a micelle has a low charge, or if there is a high salt concentration in the system, nonpolar solutes, such as hydrocarbons, are always solubilized in the interior of the micelle due to the large reduction in the interfacial energy thus produced. For systems with highly charged micelles, considerable amounts of hydrocarbons will also be solubilized in the palisade layer, near the micellar surface, lowering the surface charge density. Polar molecules are solubilized in the palisade layer by micelles with a high surface charge density, except for the first solute molecule per micelle, that is often solubilized in the interior (due to the considerable reduction in the micellar interfacial area that results). A considerable amount of polar solutes may also be solubilized in the interior of micelles with a low surface charge density.

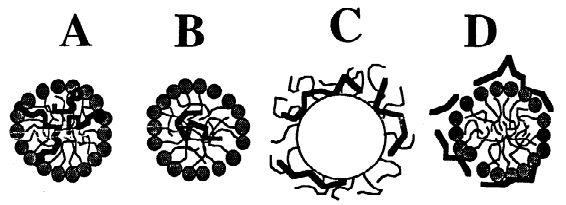

Fig. 3. Representation of the various proposed solubilization sites in a micelle. (A) Solubilization in the micellar core; (B) solubilization at the core/palisade interface; (C) solubilization in the palisade layer; and (D) adsorption at the micelle surface. (Reproduced with permission from Ref. [34]. Copyright Marcel Dekker Publishers.)

X-Ray diffraction, absorption and fluorescence, and nuclear magnetic resonance methods have been used to define the site of interaction for solutes in a micelle [18,22,29,35–37]. In the main these studies show that aromatic hydrocarbons are primarily solubilized near the micellar surface (cationic surfactants) or evenly distributed throughout the micelle (anionic surfactants). Saturated hydrocarbons are primarily located in the interior of the micelle and polar solutes at the surface. Reality is that those solutes which enter the micelle, can diffuse rapidly

within the micelle, and so experience a wide range of microenvironments. In addition, because of the size constraints of a micelle, those solutes evenly distributed throughout the micelle are statistically likely to be located close to the interface. Solute concentration and the ionic strength of the aqueous phase play an important role in site localization and assumptions used for interpretation, such as the two-state model, result in conclusions that differ between studies.

3. Conceptual model for a micellar phase in MEKC

Micelles are complex solvents and can only be treated in an approximate sense as bulk solvents. The separation buffer in MEKC contains a homogeneous dispersion of micellar aggregates distributed throughout the solution. Individual aggregates are hardly significantly larger than the solutes being separated. On account of their small size and large number, they have a high surface area-to-volume ratio. Their structures are dynamic, with the average residence time of a surfactant monomer in the micelle being on the order of 1 ms or less. Their composition is unlikely to be spatially homogeneous; the core region is hydrocarbon-like and nearly anhydrous; the surface region is polar and highly solvated by water. The size, shape and aggregation number of the micelle is dependent on its external environment, particularly the ionic strength and composition of the supporting electrolyte always present in MEKC. The surrounding electrolyte can influence selectivity in MEKC through the influence of the counterion on the sorption characteristics of the interphase region, and indirectly through its influence on micelle structure and cohesion. To this extent, the identity of buffer and surfactant counterions and their concentration, can be expected to affect the solvation properties of the micelle and the observed chromatographic selectivity. In addition, environmental factors can be expected to cause changes in general retention, through their influence on the phase ratio. Selectivity differences in MEKC resulting from the use of anionic surfactants with different cations have been observed in practice [38]. Similarly, since the effective charge on a micelle depends on the amount of electrostatically retained counterions, the above factors can influence the observed migration window in MEKC by modifying the effective micellar velocity. Jacquier and Desbene have developed a method for measuring the cmc of a surfactant by MEKC [39,40]. In the case of SDS they demonstrated significant changes in the cmc as a function of buffer concentration and composition, as well as for the concentration and type of organic solvent additives. For example, the cmc for SDS declines in an exponential fashion from about 8 to around 4 m*M* by increasing the concentration of sodium borate or sodium phosphate from 0 to 20 m*M*. The relationship with organic solvent type and concentration is more complex, showing either an increase or decrease in the cmc as the volume of organic solvent was varied, in a manner that depended mainly on solvent identity.

Normally injected analyte quantities in MEKC are small by comparison to the typical concentration of micelles in the running buffer. Each solute probably finds an individual micelle to interact with and multiple solute–micelle interactions should be uncommon. Likewise, solute–solute interactions within the solvation volume of the micelle are unlikely to influence retention and, within reasonable limits, retention should be independent of sample concentration.

In the above light, micelles have to be considered as variable solvents with properties that are impressed upon them by their external environment, augmenting their natural capacity for solvation interactions based on their structure. It cannot be ruled out that site-specific interactions result in an apparent range of solvation environments that depend on solute properties. It would seem that the task of defining the solvent properties of micelles is too complicated to comprehensively solve by any simple universal model, yet progress might be made using somewhat simple models to characterize the apparent behavior of micelles in a manner suitable for establishing their chromatographic properties.

4. Models based on linear free energy relationships for solvation by micelles

Solvation models based on free energy relationships, in our opinion, are the most appropriate

models at the moment for characterizing the solvation behavior of micelles under conditions applicable to MEKC. The Kamlet-Taft solvatochromic model was employed by Chen et al. [41] and Yang and co-workers [42–45] to determine the contribution of defined intermolecular interactions to the distribution constant (or retention factor) for several micellar systems in MEKC. The solute descriptors used in this model are not all clearly free energy-related terms (experimentally they are derived from absorption measurements of indicator compounds), and for this and other reasons detailed elsewhere [46,47], we prefer to use the solvation parameter model in the form originally suggested by Abraham [48,49]. The solvation parameter model has been used successfully in various areas of chromatography, for example, to characterize the solvent properties of stationary phases and to predict retention in gas chromatography [47,50–63], also in liquid chromatography and solid-phase extraction [63–72], and to model retention in thin-layer chromatography [73] and supercritical fluid chromatography [74]. Since we had available solute descriptors for most of the solutes used by Yang and co-workers [42–45], we have recalculated their data using the solvation parameter model, and will present those results in this article. Yang and Khaledi [43] grouped their solutes into three categories (nonpolar, hydrogen-bond acids, hydrogen-bond bases) and found substantially different microenvironments in the micellar phase for the different solute categories. Re-evaluating their data using the solvation parameter model we were unable to confirm their conclusions [75]. Inclusion of pyridine (which we regard as a wild outlier) disturbed the statistical fit of the data, and the range of values for the solute descriptors in their subsets have become compressed when the data set is divided up, resulting in unstable local fits for the data that are not general models. Yang and Khaledi [43] used parameter estimates for a number of the solute descriptors in their data set, which may have also influenced the outcome of their work.

The solvation parameter model in a form suitable for characterizing the distribution of neutral solutes between a micellar phase and a buffer in MEKC is set out below:

$$\log \mathrm{SP} = c + mV_X + rR_2 + s\pi_2^{\mathrm{H}} + a\alpha_2^{\mathrm{H}} + b\beta_2^0 \qquad (1)$$

where SP is the experimentally observed retention property (a distribution constant or the retention factor), 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_2^0 are parameters characterizing the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. Solute descriptors are available for more than 2000 compounds and others are available through parameter estimates [76]. They can be obtained by calculation (V_X and R_2) or measured experimentally in chromatographic or liquid–liquid distribution systems using standard methods [76,77]. A short list of solute descriptors useful for characterizing surfactant properties in MEKC is summarized in Table 1.

The system constants in Eq. (1) 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 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 SP for a group of solutes of known properties sufficiently varied to define all interactions in Eq. (1) and of sufficient number to establish the statistical validity of the model.

5. Distribution properties of micelle–water systems

Abraham et al. have used distribution constants for water–micelle systems to estimate the contribution of intermolecular interactions to solubility in sodium dodecyl sulfate (SDS) [78] and hexadecylpyridinium chloride (CPC) [79] micelles, and Quinn et al. [80] for micelles formed from sodium dodecyl sulfate,

Table 1
Solute descriptors for characterizing the sorption properties of micelles in MEKC

Solute	Descriptors[a]				
	V_x	R_2	π_2^H	α_2^H	β_2^0
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
n-Propylbenzene	1.1391	0.602	0.49		0.16
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
Bromobenzene	0.8914	0.882	0.73		0.09
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
Ethyl phenyl ketone	1.1550	0.804	0.95		0.51
Propyl phenyl ketone	1.2960	0.797	0.95		0.50
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
Benzyl acetate	1.2135	0.798	1.06		0.65
Methyl benzoate	1.0726	0.733	0.85		0.46
Ethyl benzoate	1.2135	0.689	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-Choroacetophenone	1.1360	0.955	1.09		0.44
1-Nitrobutane	0.8464	0.227	0.95		0.29
1-Nitropentane	0.9873	0.212	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
3-Phenylpropanol	1.1978	0.811	0.90	0.30	0.67
4-Phenylbutanol	1.3387	0.821	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
Phenylacetamide	1.1137	0.950	1.60	0.52	0.80
Benzenesulfonamide	1.0971	1.130	1.55	0.55	0.80
4-Nitroaniline	0.9910	1.220	1.91	0.42	0.38
3-Bromoaniline	0.9910	1.128	1.19	0.31	0.34
N-Ethylaniline	1.0980	0.945	0.85	0.17	0.51
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	0.860	1.35	0.69	0.45
2-Naphthol	1.1440	1.520	1.08	0.61	0.40

[a]Values are for the undissociated form and not all solutes may be useful over a wide pH range. V_x is in units of $cm^3\ mol^{-1}/100$.

hexadecyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), and the neutral poly(oxyethylene[23]dodecyl ether) surfactant Brij 35. The results are summarized in Table 2. The dominant contribution to solubility in the micelle is the more favorable cavity term (*m* constant) with a weak contribution from lone pair–lone pair electron attraction (*r* constant), while dipole-type interactions (weakly) favor solubility in water. The main difference between the anionic and cationic surfactant micelles is that the cationic micelles are strong hydrogen-bond bases with respect to water (positive *a* constant), favoring transfer of hydrogen-bond acids to the micelle. None of the micelles can compete with water as a hydrogen-bond acid, with the result that hydrogen-bond bases will have a significantly lower distribution to the micelles than other compounds. There is a significant difference between the two reported models for SDS, which is beyond statistical control, particularly for the *m* constant. There is no obvious reason for this, but in part it may arise from the use of different literature values for the distribution constants, which represent determinations by multiple methods and varying conditions. Also, the data for Brij 35 requires a note of caution in its interpretation since the number of solutes used for the model is barely adequate for a robust fit.

The above data are presented as an illustration of general trends, since water alone is never used as a mobile phase in MEKC. The general influence of the high cohesive energy and hydrogen-bond acidity of water are clearly apparent, and play a dominant role in the distribution process. These trends are expected to persist in electrolyte media more typical of MEKC, but could be modified to the extent that the micelle adapts to the external electrolyte solution through changes in its dimensions and cohesion.

6. Distribution properties of micelle–electrolyte systems in MEKC

Poole and Poole [81] have determined the system constants for the surfactants SDS, sodium N-dodecanoyl-N-methyltaurine (SDMT), sodium cholate (SC), sodium deoxycholate (SDC), sodium taurocholate (STC), sodium taurodeoxycholate (STDC) by MEKC in a 10 m*M* each sodium borate and sodium phosphate buffer (pH 8) at 25°C (Table 3). Results are also presented for hexadecyltrimethylammonium bromide (CTAB) in a 20 m*M* sodium phosphate buffer (pH 7) at 25°C. From the data of Yang and Khaledi [43], we have calculated further values for SDS, SC, lithium perfluorooctanesulfonate (LPOS), and tetradecyltrimethylammonium bromide (TDTAB) in a 50 m*M* sodium phosphate buffer (pH 7) at 25°C. In addition, from the data presented by Herbert and Dorsey [82] we have calculated a further parameter set for SDS in a 100 m*M* sodium borate and 60 m*M* sodium phosphate buffer (pH 7) at 30°C. Other literature sources lack either a sufficient number of solutes or variety of solutes to be useful in developing models of the solvation properties of additional micellar systems. We note in passing for the data in Table 3, the overall correlation coefficient exceeds 0.984 (and is generally higher than 0.990), the standard error in the estimate of $\log k$ is <0.2 (and is generally <0.1), and the F statistic is >100 (typically 300–600). The models in Table 3 are

Table 2
System constants for water–micelle distribution systems

Surfactant	System constants					No. of solutes
	m	*r*	*s*	*a*	*b*	
SDS[a]	2.79	0.54	−0.40	−0.13	−1.58	132
SDS[b]	3.25	0.32	−0.57	−0.08	−1.84	66
CPC	3.39	0.97	−0.74	0.77	−2.84	46
CTAB	3.57	0.76	−0.32	1.02	−3.78	42
DTAB	2.98	0.57	−0.40	0.28	−1.82	39
Brij 35	3.65	1.63	−0.37	1.62	−3.83	19

[a]Data from Ref. [78].
[b]Data from Ref. [80].

Table 3
System constants for aqueous buffer–micelle distribution systems determined by MEKC

Surfactant	Conc. (m*M*)	System constants						No. of solutes	Ref.
		m	*r*	*s*	*a*	*b*	*c*		
SDS	50	2.99	0.46	−0.44	−0.33	−1.88	−1.82	40	[81]
	50	2.91	0.31	−0.24	−0.44	−1.87	−1.85	32	[82]
	20	2.81	0.38	−0.28	−0.16	−1.80	−2.18	47	[43]
SC	50	2.59	0.65	−0.47	0	−2.27	−2.11	40	[81]
	75	2.45	0.63	−0.47	0	−2.29	−1.71	40	[81]
	125	2.39	0.48	−0.46	0	−2.14	−1.34	40	[81]
	60	2.41	0.57	−0.55	0	−2.45	−1.60	47	[43]
	80	2.63	0.40	−0.60	0	−2.59	−1.47	47	[43]
SDC	75	2.67	0.66	−0.47	0	−2.47	−1.69	40	[81]
STC	50	2.43	0.60	−0.34	0	−2.06	−2.10	40	[81]
STDC	50	2.62	0.67	−0.45	0	−2.17	−1.99	40	[81]
SDMT	50	3.07	0.72	−0.50	0.22	−2.58	−2.01	40	[81]
LPOS	40	2.28	−0.54	0.48	−0.89	−0.60	−2.05	47	[43]
CTAB	50	3.40	0.61	−0.55	0.58	−3.08	−1.67	36	[81]
TDTAB	10	2.76	0.28	0	0.94	−2.62	−2.09	47	[43]

therefore statistically sound as well as being chemically sensible.

Given allowance for the range of electrolyte and surfactant concentrations at which individual experiments were performed, there is good general agreement for the solvation properties determined for SDS and SC in Table 3. The large differences in the model constant (*c* term) with concentration, are expected, since this term contains the value for the phase ratio. For SDS and SC the primary change in retention with increasing concentration, at least for the conditions represented by the data in Table 3, is a general increase in retention due to a decrease in the phase ratio. Within the range of the experimental data, changes in selectivity accompanying either changes in the electrolyte concentration or surfactant concentration, for SDS or SC, cannot be unequivocally discerned. However, as the range of values for the system constants across all experimental conditions for SDS and SC in Table 3, is itself not large, then presumably neither is the general influence of experimental conditions on the selectivity of these two micellar systems. Similar conclusions were reached in a somewhat more methodical study of the influence of experimental parameters on the solvation properties of SC for reasonable operating conditions in MEKC [81].

In terms of solvent selectivity, the bile salt surfactants in Table 3 (SC, SDC, STC, and STDC) are most alike as a group, while at the same time different in sorption characteristics to the other surfactants shown. Choosing surfactants from within this group can only be expected to provide small changes in selectivity (most notably in the separation of hydrogen-bond bases), as observed in the chromatograms from test mixtures [81]. Albeit that considerable care is needed in the interpretation of selectivity from the separation of test mixtures, because the common presumption that dominant intermolecular interactions can be associated with individual solutes, is generally not tenable. Nearly all compounds possess a number of polar characteristics that vary in intensity, and the dominance of a single characteristic is unusual, rather than the norm, as can be seen from the collection of solute descriptors in Table 1. The importance of a particular solute characteristic also depends on the availability of complementary solvent properties. For example, the comigration of anisole and methyl 3-hydroxybenzoate in SC is due to compensation of the stronger dipole-type ($s\pi_2^H$) and hydrogen-bond ($b\beta_2^0$) interactions of methyl 3-hydroxybenzoate with the aqueous buffer being offset by differences in the cavity term for the two solutes (Table 4). The fact that methyl 3-hydroxybenzoate is a significantly stronger hydrogen-bond acid than anisole is irrelevant because the hydrogen-bond basicity of the micellar phase and aqueous buffer are equal (*a*

Table 4
Contribution of intermolecualr interactions to the separation of anisole and methyl 3-hydroxybenzoate by MEKC

Surfactant (mM)	Solute[a]	Intermolecular interaction						Retention factor (k)
		mV_X	rR_2	$s\pi_2^H$	$a\alpha_2^H$	$b\beta_2^0$	c	
SC (75)	Anisole	2.24	0.45	−0.35		−0.66	−1.71	0.92
	MHB	2.77	0.57	−0.66		−1.03	−1.71	0.87
SC (125)	Anisole	2.19	0.34	−0.35		−0.62	−1.34	1.67
	MHB	2.70	0.43	−0.64		−0.96	−1.34	1.55
SDMT (50)	Anisole	2.81	0.51	−0.38		−0.75	−2.01	1.55
	MHB	3.47	0.65	−0.70	0.15	−1.61	−2.01	2.51

[a]MHB, methyl 3-hydroxybenzoate.

constant is zero). Consequently, peak positions in a chromatogram are the result of a balance of intermolecular interactions combined with size differences. A powerful feature of the solvation parameter model, is its usefulness in dissecting retention information into intermolecular interactions, that provides fundamental insight into the retention mechanism that is absent from simply noting the peak positions in a series of chromatograms.

SDS has different selectivity to the bile salt surfactants; it is slightly less cohesive (larger m constant), a much weaker hydrogen-bond base (negative a constant) and a stronger hydrogen-bond acid (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. SDMT is a significantly stronger hydrogen-bond base (positive a constant), weaker hydrogen-bond acid (large negative b constant), and is less cohesive (larger m constant) than the bile salts and SDS. As well as a higher general retention of weakly polar solutes, the greatest difference in migration order is expected for hydrogen-bond-forming solutes. These are properties that favor the separation of anisole and methyl 3-hydroxybenzoate, as shown in Table 4, and illustrates the use of the solvation parameter model for selecting surfactant systems for achieving individual separations. LPOS is the most cohesive of the surfactant micelles in Table 3 (smallest m constant), is the most competitive with the aqueous buffer as a hydrogen-bond acid (smallest negative b constant), is considerably more dipolar in character than the other micelles (positive s constant), and is a very weak hydrogen-bond base (largest negative a constant). The negative r constant is characteristic of fluoroalkane compounds in general, representing their lower polarizability compared to similar hydrocarbon compounds. Since the perfluorooctanesulfonate group has no available protons to act as hydrogen-bond acids, we can only speculate that its hydrogen-bond acidity arises from the inductive effect of fluorine on water molecules in contact with the sulfonate group. Alternatively, the hydrogen-bond acidity may be a property of differences between the hydrated counterions (lithium compared to sodium) bound in the interphase region of the micelle, as suggested by Abraham et al. [79], to explain the difference in hydrogen-bond acidity between SDS and CPC micelles. The characteristic feature of the cationic micelles (CTAB and TDATB) is their strong hydrogen-bond basicity (positive a constant) and weak hydrogen-bond acidity (large negative b constant).

From the perspectives of methods development in MEKC, it would seem desirable to have at hand a more varied collection of surfactants than those indicated in Table 3. Yet the surfactants identified in Table 3 represent those most commonly used in contemporary MEKC practice. The largest selectivity variation for the surfactants in Table 3 is observed for hydrogen-bond acids and bases. The range of cohesive properties and capacity for dipole-type interactions is narrow. In particular, there is a close grouping among the anionic surfactants if LPOS is removed from consideration. It is impossible to theorize what range of selectivity might be available for other surfactant systems in the absence of experimental data, since the relationship between surfactant structure and micelle solvation characteristics has not developed so far. This is an obstacle that can be overcome by using the solvation parameter

model as a tool to characterize the sorption properties of micelles.

It is possible to overlay the solvation properties of other water-containing distribution systems on the data in Table 3 to arrive at some ideas for a possible working range of micellar solvation properties. For water-immiscible organic solvent–water distribution systems, typical values for the system constants are $m = 2.78–4.70$; $r = -0.25–1.60$; $s = 0$ to -1.75; $a = 0.1$ to -3.80; and $b = -2.25$ to -5.00 [76]. Typically, the micellar systems are concentrated at the low end of the solvent m scale; clustered around the middle of the r scale; clustered in the upper portion of the solvent s scale; at the top of the solvent a scale (several micelles are stronger hydrogen-bond bases than the water-immiscible organic solvents); and clustered in the upper portion of the solvent b scale. In this sense, the solvation behavior of the surfactant micelles in Table 3 would be considered most similar in properties to water-saturated alcohols, and are thus quite polar. LPOS would be considered an atypical solvent based on this comparison to 23 water-immiscible organic solvents, and the cationic surfactants as stronger hydrogen-bond bases than any of the water-immiscible alcohols.

Chemically-bonded, stationary phases in reversed-phase liquid chromatography could be considered as interfacial solvents with properties perhaps closer to those of micelles than those of bulk solvents. For comparison some system constants for these solvated sorbents using a nearly totally aqueous mobile phase, the most meaningful for a comparison, are summarized in Table 5 [64–66]. There are clear similarities and differences between the data in Tables 3 and 5. The bonded-phase sorbents show a much wider range of cohesion (m constant) and a wider range of hydrogen-bond acidity (b constant) if LPOS is excluded. The micelles are stronger hydrogen-bond bases, in the main, and slightly less dipolar, in general. Since the m and b constants are generally those with the largest effect on retention characteristics when one phase is water, the narrower range for these constants, exhibited by the micellar phases, suggests an obvious possibility for increased variation in their solvation properties, through synthesis, or identification of additional surfactants with the desired characteristics. In general, the whole of the selectivity space cannot be explored with the surfactants in Table 3, providing the initiative for a more organized and systematic study of surfactant solvation properties under conditions germane to MEKC. In this endeavor, the solvation parameter model could play a pivotal role in identifying surfactants with (near) duplicate properties, so that eventually a sensible number of surfactants with varied properties are available for methods development.

Table 5
System constants for reversed-phase liquid chromatographic systems with water containing 1% (v/v) methanol as the mobile phase

Sorbent[a]	System constants				
	m	r	s	a	b
PSP	5.44	0.93	−0.63	−1.45	−3.44
C_{18} (HL)	5.65	0.70	−0.76	−0.40	−3.26
C_{18} (LL)	3.92	0	−0.11	−0.54	−1.53
C_4	3.36	0	0	−0.46	−1.53
CN	2.06	0.53	0	−0.51	−1.45
DIOL	1.57	0.61	0	−0.45	−0.80

[a]PSP, Polymer Laboratories PLRP-S 300 (a styrene–divinylbenzene porous polymer); C_{18} (HL), Bakerbond octadecylsiloxane-bonded silica with a high loading; C_{18} (LL), Bakerbond octadecylsiloxane-bonded silica with a low loading; C_4, Bakerbond WP butylsiloxane-bonded silica; CN, Bakerbond cyanopropylsiloxane-bonded silica; and DIOL, Bakerbond spacer-bonded propanediol siloxane-bonded silica.

7. Interphase model for retention by micelles in MEKC

Progress in a scientific method is often guided by conceptual models that in their infancy turn out to be too crude or simple to divulge the complete picture. Most of our understanding of retention in MEKC has been guided by assuming that an interphase model is adequate to explain selectivity differences resulting from solute–micelle interactions. We define the interphase as that region surrounding the core of the micelle containing the polar head groups and possibly immediate neighbor segments of the surfactant tail, as well as components of the electrolyte solution, organized into a loose structure on account of their proximity and attraction to the micelle interface. The actual boundaries between the core of the micelle and the interphase region, and the interphase

region and the bulk electrolyte solution, are not well defined, and may change as the electrolyte composition is varied. The composition of the interphase may not be homogeneous, but as the interphase layer is thin, solutes can readily explore all regions, resulting in an averaging effect when macroscopic properties are determined. The electrolyte composition in the interphase is probably different to that of the bulk solvent, and is controlled by short-range surface electrostatic forces; similarly, the composition of the interphase may be different to the bulk electrolyte when organic additives are present in the mobile phase, due to selective solvation of the micelle surface groups by the additive. Surfactant molecules must be able to enter and leave through the interphase region to maintain the dynamic nature of the micelle structure. Retention results from the difference in solvation characteristics of the interphase region to those of the bulk electrolyte solution. It is possible that the core of the micelle does not play a significant role in retention beyond its role in forming the micelle and responding to changes in the micelle structure imposed on the micelle by its external environment.

Supporting evidence for the interphase model of solvation in MEKC comes from several sources. The number of solute molecules with respect to the number of micelle aggregates is low, so that there is no mass balance effect to force deeper penetration into the micelle. It is likely that the solute, in fact, has a minimal impact on micellar properties at typical concentrations employed in MEKC. The solute environment in the micellar phase is very polar as indicated by the comparison of surfactant–micelle system constants to those for water-immiscible organic solvents. It is also at least partly aqueous, as indicated by the significant hydrogen-bond acidity of the anionic micelles, which lack suitable protons within their structure for this purpose. The retention data for varied solutes (Table 1) is homogeneous with respect to the construction of the solvation parameter models, suggesting a uniform average solvation environment for all solutes. Some distortion in the model fit might have been expected for solutes favoring the hydrocarbon core region over the polar interfacial region of the micelle (although it could be argued that because of the small size and rapid diffusion of solutes within the micelle aggregates that an averaging effect would prevail).

Additional support for the interphase model was sought from studies of mixed surfactant micelles, and the influence of organic solvent additives on retention in MEKC [83,84]. The variation of the system constants for the mixed surfactant system containing SDS and various amounts of the nonionic surfactant Brij 35 in the mole ratio from 0 to 1, is shown in Fig. 4 (similar results were obtained in the same concentration range for SDMT and Brij 35). The addition of Brij 35 to SDS causes only small changes in the *m*, *r*, and *s* constants; a slight increase in the hydrogen-bond basicity of the mixed surfactant micelle (*a* constant); and at low concentrations a significant change in the hydrogen-bond acidity of the mixed surfactant micelle (*b* constant) which eventually flattens out at higher molar ratios. NMR evidence suggests that at low incorporation of Brij 35 into SDS the hydrophobic core of the mixed micelle is little perturbed by the presence of Brij 35, which seems to be localized in the vicinity of the polar head groups [85]. Localization of Brij 35 in the interphase region at low mole ratios would explain the diminished capacity of the mixed surfactant micelles to function as hydrogen-bond acids due to

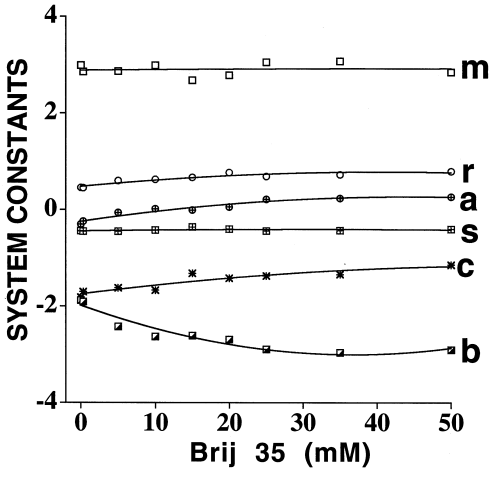

Fig. 4. Plot of the system constants derived from the solvation parameter model against composition of the mixed surfactant buffer containing 50 m*M* SDS and 0–50 m*M* Brij 35 in 10 m*M* each sodium tetraborate and sodium phosphate buffer (pH 8) at 25°C. (Reproduced with permission from Ref. [84]. Copyright Dr. Alfred Huethig Publishers.)

dilution and intermolecular hydrogen bonding with the hydrogen-bond basic poly(ether) surfactant. At higher mole ratios of Brij 35 we speculate that most of the nonionic surfactant is stored in the core of the mixed surfactant micelle, or at least away from the interphase region, where it no longer significantly influences the solvation properties of the interphase region. Brij 35 is known to form rod-like micelles with the poly(oxyethylene) chains wrapped around the central core of alkane chains with a dilute corona of protrusions rising above the core [86]. We envision a model for the mixed surfactant micelle something like that shown in Fig. 3C with the outer layer of the micelle made up of the polar portion of SDS and some fraction of the poly(ether) chains of Brij 35. Adding Brij 35 to the SDS surfactant causes an expansion of the micelle without significant change in the surface composition of the micelle, which in turn maintains a relatively constant composition for the interphase region. The cohesive energy of the interphase region should not be significantly influenced by the entry of the nonionic surfactant into the core of the ionic micelle, and the small change in hydrogen-bond acidity of the interphase region are accounted for by the selective intermolecular interactions of hydrogen-bond acid groups associated with water molecules in the region of the sulfonate head groups of the micelle, with the hydrogen-bond base centers of the poly(ether) surfactant protruding into the interphase region. The smooth change in the phase ratio (contained in the *c* term in Fig. 4), and the effective mobility of the mixed surfactant micelle as a function of composition, combined with a distinct and single peak for elution of the mixed micelles in MEKC, add support to the assumption that a continuous macrostructure is formed for all compositions investigated.

The variation of the system constants for a mixed surfactant buffer containing SDMT and Brij 35 (5:2) to which various volume fractions of acetonitrile are added is shown in Fig. 5 [83]. Increasing the volume fraction of acetonitrile has two obvious effects; it reduces retention by lowering the difference in cohesive energy between the interphase region and the bulk electrolyte solution, and by increasing the hydrogen-bond acidity of the interphase region relative to that of the bulk electrolyte solution. These results are reminiscent of those observed for chemically bonded stationary phases in reversed-phase liquid chromatography for the same range of mobile-phase composition [64,65]. At a fixed volume fraction the identity of the solvent (acetonitrile, methanol, isopropanol, or tetrahydrofuran) seems to be less important than its volume fraction in modifying the system selectivity for the mixed surfactant micelles, or for SC micelles [81], when compared to the changes observed in reversed-phase liquid chromatography. The addition of organic solvent to the mixed surfactant–micelle system results in the uptake of organic solvent by the interphase region relative to the composition of the bulk electrolyte solution, probably due to a combination of selective solvation of the micelle interface and the solvophobic effect of water (there is little evidence to support the uptake of low-molecular-weight organic solvents by the micelle core [26,87,88]). For predominantly aqueous systems, which is the general case for MEKC, the composition difference between the interphase and the bulk electrolyte solution is not great, such that the changes in selectivity observed for the four organic solvents are small. The main difference between the totally aqueous electrolyte and the solvent-containing electrolyte solution is probably due to solvent disruption of the water

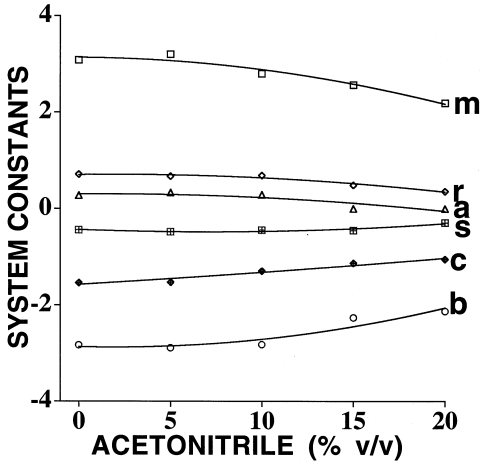

Fig. 5. Plot of the variation of the system constants with volume fraction of acetonitrile added to a mixed micellar buffer containing 50 m*M* SDMT, 20 m*M* Brij 35, 10 m*M* sodium borate, and 10 m*M* sodium phosphate. The pH of the aqueous solution before addition of organic solvent was 8. (Reproduced with permission from Ref. [83]. Copyright The Royal Society of Chemistry.)

structure accompanied by selective solvent–water interactions. Although the range of organic solvent composition variation is much smaller in MEKC than can be achieved in reversed-phase liquid chromatography, due to instability of the micelle aggregates at moderate (e.g. >25%) volume fractions of low-molecular-weight organic solvents, the change in system constants (Fig. 5) is sufficiently useful to exploit in methods development. Since the relationships between the system constants and the volume fraction of organic solvent are smooth, the outcome in methods development can be predicted from plots such as Fig. 5.

8. Conclusions

A general understanding of the retention properties of micelles in MEKC must take into account the dynamic nature of surfactant aggregates and their changing properties imposed on them by their external environment. The dominant feature of their solvation properties is their large surface-to-volume ratio, small dimensions, and spatial heterogeneity. The capacity of surfactant micelles for varied solute interactions in MEKC can be characterized through the solvation parameter model, which in turn, provides a connection to the solvation properties of other chromatographic systems. Analogies with chemically bonded stationary phases for reversed-phase liquid chromatography indicate that the surfactants commonly used in MEKC provide only a restricted range of selectivity variation for methods development, as well as highlighting the complementary properties of the two separation techniques (some micelles are significantly stronger hydrogen-bond bases than common chemically bonded phases). An interphase model is introduced and shown to be capable of explaining the retention properties of micelles under MEKC conditions.

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