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Characterization of chemical selectivity in micellar electrokinetic chromatography
VI. Effects of surfactant counter-ion

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Abstract

Linear solvation energy relationships and free energy of transfer data were used to evaluate the influence of the surfactant counter-ion on selectivity in micellar electrokinetic chromatography. It was determined that selectivity differences are dependent on the valency of the counter-ion but not the type of counter-ion. Monovalent surfactants, sodium dodecyl sulfate (SDS) and lithium dodecyl sulfate, have nearly identical selectivity behavior. The divalent surfactants, magnesium didodecyl sulfate and copper didodecyl sulfate also show very similar behavior. However, when the divalent counter-ion species is compared to SDS under similar conditions, significant differences are observed. Most notably, the utilization of divalent counter-ion species of dodecyl sulfate surfactants causes the micelles to become more hydrophobic and a weaker hydrogen bond donating pseudo-stationary phases. It is believed that the divalent counter-ions reduce the electrostatic repulsion between the surfactant head groups and therefore, increase the chain packing of the monomers in the micelle aggregates. This reduces the degree of hydration of the micellar palisade layer leading to a decreased ability of the micelle to participate in polar/polarizable and hydrogen bonding interactions with solute molecules.

Keywords: Selectivity; Linear solvation energy relationships; Micellar electrokinetic chromatography; Surfactants

1. Introduction

Since its introduction, micellar electrokinetic chromatography (MEKC) has proved to be a widely useful separation technique [1,2]. It expands the utility of capillary electrophoresis because of its ability to separate uncharged solutes via the differential partitioning between the bulk aqueous phase and a micellar pseudo-stationary phase. The primary driving force for this partitioning is hydrophobic interactions between the solute and micelles. However, as several groups have discussed, there are other more specific interactions (e.g. dipole–dipole, dipole–induced dipole, and hydrogen bonding) that can have a significant influence on selectivity in MEKC [3–9]. Using linear solvation energy relationships (LSERs), these studies reported striking differences in selectivity between hydrocarbon, fluorocarbon, bile salt, anionic, and cationic surfactants as well as polymer and mixed micellar phases. Some of these reports [3–6,9] used the LSERs based on

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studies by Kamlet, Taft, and their co-workers describing solvation effects on physicochemical processes [10,11]. Others [7,8,14] have used a modified model and the more reliable solute solvation parameters described by Abraham et al. [12,13]. However, the understanding of the fundamental differences in selectivity between surfactants in MEKC is independent of the LSER model used to determine the selectivity parameters [14].

In many of the previous reports, the micellar systems that were studied varied greatly. Some research has looked at the effect of changing various separation conditions such as pH, buffer and surfactant concentration, temperature, as well as the addition of organic modifiers [8,15,16]. Less attention has been given to structurally similar surfactants and the effects that small changes have on the observed selectivity. In a series of manuscripts, this group has investigated these effects by systematically changing various structural properties of the surfactant monomers that are used to form the MEKC separation media [17–19]. In these reports, it was concluded that the degree of hydration near the palisade and Stern layers plays a very significant role in the hydrogen bonding and polarity characteristics observed by solutes that partition into the micellar phase. This is evident by the large influence the surfactant head group can have on selectivity in MEKC. It was also determined that the hydrocarbon chain length has a small contribution depending on the nature of the head group in the surfactant homologous series.

To obtain a more complete understanding of the role of surfactant structure on chemical selectivity, this report expands on those studies by investigating the influence of the surfactant counter-ion. Since they are present in a relatively high concentration at the micelle surface, it was hypothesized that the type of counter-ion may have a notable influence on the amount of the water present in palisade and Stern layers of the micelle and, therefore, on selectivity in MEKC. Several workers have discussed some of the effects of monovalent counter-ions on MEKC properties. Muijselaar et al. used LSERs to compare the selectivity differences between the sodium and TRIS counter-ions of dodecyl sulfate and concluded that these two systems have the same selectivity characteristics [16]. Ahuja and Foley compared the lithium, sodium, and potassium counter-ion effects on efficiency and resolution in MEKC using dodecyl sulfate surfactants and noted that lithium dodecyl sulfate (LiDS) and sodium dodecyl sulfate (SDS) have very similar selectivity properties in the absence of organic modifiers [20]. However, after the addition of various amounts of acetonitrile, they observed that the counter-ion did influence the selectivity. In a similar study, which used the lithium, sodium and potassium salts of dodecoxycarbonyl valine surfactants, which possesses a more organic head group, the counter-ion influence was more significant [21].

All of the surfactant systems in this report consist of the same hydrophobic tail (dodecyl hydrocarbon chain) and headgroup (sulfate), and only differ in the type of inorganic counter-ion. Two monovalent counter-ions (lithium and sodium) and two divalent counter-ions (magnesium and copper) were evaluated. Nielson and Foley investigated the influence of magnesium on selectivity in MEKC previously [23]. However, they also used 4 m*M* EDTA and varying concentrations of acetonitrile in the buffer solution. To avoid potential competitive interactions, no buffer additives were included in the divalent counter-ion solutions in this study. As in previous MEKC studies from our group, LSERs and free energy of transfer studies ($\Delta\Delta G$) have been utilized to compare the different systems. The LSER results for SDS have been reported previously, but have been included here for comparison since it is the most commonly used surfactant for MEKC separations.

2. Experimental

2.1. CE apparatus

All MEKC experiments were performed on a laboratory built CE system equipped with a 0–30 kV power supply (Series EH, Glassman High Voltage, Whitehouse Station, NJ, USA), an Acutect 500 variable-wavelength UV–Vis detector, and a Hewlett-Packard HP3394A integrator. A 58 cm (effective length 42 cm)×50 μm I.D.×375 μm O.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used. A voltage of 25 kV was applied throughout the experiments.

2.2. Preparation of divalent species

Magnesium didodecylsulfate [$Mg(DS)_2$] and copper didodecyl sulfate [$Cu(DS)_2$] were made by first dissolving SDS in doubly distilled water in two different Erlenmyer flasks. Sufficient amounts of either $MgCl_2$ or $CuCl_2$ were added to separate flasks in order to make the DS^-/X^{2+} ratio slightly less than 2/1. Each solutions was stirred for 30 min at about 30°C before cooling in an ice water bath to form the $Mg(DS)_2$ and $Cu(DS)_2$ precipitates. The solids were then filtered and rinsed with ice water. The dissolution and recrystallization process was repeated three times for each surfactant. The resulting solids were left to dry for over 24 h. By monitoring the sodium atomic emission line intensity (Perkin-Elmer 3110 atomic emission spectrometer), the sodium content was found to be less than 1% (w/w) for each of the of the divalent surfactant species.

2.3. Micellar buffer solutions

SDS and LiDS (Aldrich) were used as received. Each of the running buffers was 40 m*M* surfactant and 10 m*M* phosphate buffer (pH 7). The surfactants were dissolved in 2 ml of a 50 m*M* phosphate buffer (pH 7) stock solution before being quantitatively transferred and diluted to 10 ml with Milli-Q water. To minimize the amount of Na^+ present in the solution, the phosphate stock buffer used for LiDS was made by titrating a 50 m*M* phosphoric acid solution with LiOH in order to bring the buffer to pH 7. The separation conditions for the LiDS and SDS comparison were performed at 25°C.

The LSER experiments for $Mg(DS)_2$ and $Cu(DS)_2$ were performed using 40 m*M* of each surfactant in the absence of buffer. Buffer was not used in these solutions to maintain a single cation in the solution and to avoid introducing another (e.g. Na^+), which may obscure the divalent counter-ion effects. The pH values of these solutions were 4.2 and 4.3, respectively. For comparison, an appropriate amount of SDS was dissolved in exactly 2 ml of 50 m*M* sodium acetate buffer, pH 4.5, stock solution before being quantitatively transferred and diluted to 10 ml with Milli-Q water in order to make a 40 m*M* SDS, 10 m*M* acetate running buffer. The separation temperature for all three of these systems was maintained at 35°C because the Krafft points for $Mg(DS)_2$ and $Cu(DS)_2$ are slightly above room temperature.

2.4. MEKC samples and conditions

All test solutes (Aldrich) were dissolved in methanol [electroosmotic flow (EOF) marker] with dodecanophenone (micelle marker). The solutes were introduced to the separation capillary by a 3-s hydrodynamic injection and detected at 254 nm. The retention of the test solutes was determined in triplicate for each surfactant using Eq. (1):

$$k' = \frac{(t_r - t_{eo})}{t_{eo}\left(1 - \frac{t_r}{t_{mc}}\right)} \quad (1)$$

In this equation, t_r is the solute retention time, t_{eo} is the EOF elution time (methanol), and t_{mc} is the micellar elution time (dodecanophenone).

3. Results and discussion

3.1. Linear solvation energy relationship and free energy of transfer models

The LSER model (Eq. (2)), involves regressing the experimentally measured retention factor of a set of test solutes against the solutes' known solvation parameter values:

$$\log k' = c + mV_x + s\pi^*_2 + rR_2 + b\Sigma\beta_2 + a\Sigma\alpha_2 \quad (2)$$

In this equation, the solute parameters are given by V_x, π^*_2, R_2, $\Sigma\beta_2$, and $\Sigma\alpha_2$. V_x represents the McGowan characteristic volume of the solutes [22]. This term is divided by 100 in order to bring it to scale with the other descriptors. The polarity/polarizability of the solute is described by π^*_2. R_2 describes the solutes' excess molar refraction and is divided by 10 to roughly bring it to scale. The solute hydrogen bonding properties are described by the last two terms, where $\Sigma\beta_2$ represents the solute hydrogen bond accepting ability and $\Sigma\alpha_2$ is the solute hydrogen bond donating ability. The subscript 2 simply shows that these are solute descriptor values.

The coefficients of these parameters give a relative measure of the importance of each type of interaction that the micelles have with the solutes. The cavity formation and dispersive interactions of the micelles are described by *m*, and the dipolarity/polarizability of the micelles are characterized by *s*. The rR_2 term has been described as a polarization correction term for the model, and therefore, *r* represents a degree of polarizability that is not accounted for by the $s\pi^*_2$ term. Finally, the hydrogen bond donating and accepting ability of the micelle is represented by *b* and *a*, respectively. The system constant, *c*, contains information about the model that is not explained by the other parameters. When Eq. (2) is used, the phase ratio of the separation media is the most significant contributor to the constant, *c*.

The solutes used for the LSER analysis and their solvation parameters are listed in Table 1. Although they possess a wide range of sizes and polarities, they have been roughly classified based on their hydrogen bonding ability. Solutes with $\Sigma\beta_2$ values ≤ 0.22 are considered to be non-hydrogen bonding solutes (Nos. 1–12 in Table 1). Solutes with $\Sigma\beta_2$ values ≥ 0.22 and greater than their α values are

Table 1
Test solutes and their solvation descriptors[a]

	Solutes	V_x	π^*_2	R_2	$\Sigma\beta_2$	$\Sigma\alpha_2$
1	Benzene	0.716	0.52	0.610	0.14	0.00
2	Toluene	0.857	0.52	0.601	0.14	0.00
3	Ethylbenzene	0.998	0.51	0.613	0.15	0.00
4	Propylbenzene	1.139	0.50	0.604	0.15	0.00
5	*p*-Xylene	0.998	0.52	0.613	0.16	0.00
6	Chlorobenzene	0.839	0.65	0.718	0.07	0.00
7	Bromobenzene	0.891	0.73	0.882	0.09	0.00
8	Iodobenzene	0.975	0.82	1.188	0.12	0.00
9	4-Chlorotoluene	0.980	0.67	0.705	0.07	0.00
10	Biphenyl	1.324	0.99	1.360	0.22	0.00
11	Naphthalene	1.085	0.92	1.360	0.20	0.00
12	1-Methylnaphthalene	1.226	0.90	1.344	0.20	0.00
13	Acetophenone	1.014	1.01	0.818	0.48	0.00
14	Benzonitrile	0.871	1.11	0.742	0.33	0.00
15	Nitrobenzene	0.891	1.11	0.871	0.28	0.00
16	Methyl benzoate	1.073	0.85	0.733	0.46	0.00
17	Ethyl benzoate	1.214	0.85	0.689	0.46	0.00
18	4-Chloroanisole	1.038	0.86	0.838	0.24	0.00
19	4-Nitrotoluene	1.032	1.11	0.870	0.28	0.00
20	4-Chloroacetophenone	1.136	1.09	0.955	0.44	0.00
21	Methyl-2-methylbenzoate	1.214	0.87	0.772	0.43	0.00
22	Phenyl acetate	1.073	1.13	0.661	0.54	0.00
23	3-Methylbenzyl alcohol	1.057	0.90	0.815	0.59	0.33
24	Phenethyl alcohol	1.057	0.83	0.784	0.66	0.30
25	Benzyl alcohol	0.916	0.87	0.803	0.56	0.33
26	Phenol	0.775	0.89	0.805	0.30	0.60
27	4-Methylphenol	0.916	0.87	0.820	0.31	0.57
28	4-Ethylphenol	1.057	0.90	0.800	0.36	0.55
29	4-Fluorophenol	0.793	0.97	0.670	0.23	0.63
30	4-Chlorophenol	0.898	1.08	0.915	0.20	0.67
31	4-Bromophenol	0.950	1.17	1.080	0.20	0.67
32	4-Chloroaniline	0.939	1.13	1.060	0.31	0.30
33	3-Chlorophenol	0.898	1.06	0.909	0.15	0.69
34	3-Methylphenol	0.916	0.88	0.822	0.34	0.57
35	3-Bromophenol	0.950	1.15	1.060	0.16	0.70
36	3,5-Dimethylphenol	1.057	0.84	0.820	0.36	0.57

[a] Solute descriptors from Ref. [30].

classified as hydrogen bond acceptors (Nos. 13–25). Finally, hydrogen bond donors are those solutes with α values greater than their $\Sigma\beta_2$ values (Nos. 26–36).

Comparing the difference in free energy of transfer for different functional groups is also used to characterize each MEKC pseudo-stationary phase. The functional group selectivity, τ, can be defined as the ratio of retention factors between a mono-substituted benzene and benzene [i.e. k'(Bz–R)/k'(Bz)] [3]. The difference in free energy of transfer of a functional group from the aqueous phase to the micellar phase, $\Delta\Delta G$, can then be determined using Eq. (3):

$$\Delta\Delta G = -RT \ln \tau \quad (3)$$

If the functional group leads to more favorable interaction (e.g. longer retention) with the micelles relative to benzene, $\Delta\Delta G$ will be negative. If the addition of a functional group reduces the degree of micelle–solute interaction, $\Delta\Delta G$ will be positive. Comparing $\Delta\Delta G$ for different micellar phases, the more negative (or less positive) $\Delta\Delta G$, the stronger the interaction between the solute and that surfactant.

3.2. Comparison of monovalent inorganic counter-ions

The LSER results for all of the surfactant systems studied are listed in Table 2. A comparison between the two monovalent systems suggests that there is a very small selectivity difference between using Li^+ and Na^+ as the counter-ion. The 'organic nature' (*m* coefficient in Table 2) and hydrogen bonding properties (*a* and *b*) of these two systems show that they have almost identical selectivity behavior based on these types of interactions. The only notable distinction is the polarity/polarizability terms of these systems. The *s* and *r* coefficients for SDS suggest that it has marginally stronger interactions with polar/polarizable solutes than LiDS. However, this difference is statistically questionable and is not observed experimentally. The free energy of transfer difference ($\Delta\Delta G$) for these systems also shows that they are quite similar (Table 3).

The reports of Muijselaar et al. [16], Ahuja and Foley [20] and Peterson and Foley [21] concluded that in most cases changes in monovalent counter-ions had little effect on selectivity. One exception to this observation was when the surfactant contained a larger, more organic head group [21]. This suggests that the degree to which the counter-ion influences selectivity also depends on other structural aspects of the surfactant (e.g. head group). Peterson and Foley noticed that changing the surfactant counter-ion had a larger influence on the observed selectivity for the more hydrophobic surfactant dodecylcarbonylvaline

Table 2
Surfactant counter-ion effect on migration behavior in MEKC[a]

Surfactant	*c*	*m*	*s*	*r*	*b*	*a*	R^2
SDS[b]	−1.80	2.95	−0.30	0.19	−1.84	−0.17	0.985
		(0.11)	(0.08)	(0.07)	(0.09)	(0.04)	
LiDS[b]	−1.85	3.01	−0.37	0.31	−1.79	−0.20	0.985
		(0.11)	(0.08)	(0.08)	(0.09)	(0.05)	
SDS[c]	−1.85	2.85	−0.31	0.26	−1.70	−0.15	0.989
		(0.09)	(0.06)	(0.06)	(0.07)	(0.04)	
SDS[d]	−1.87	2.90	−0.26	0.17	−1.70	−0.18	0.988
		(0.09)	(0.07)	(0.06)	(0.08)	(0.04)	
$Mg(DS)_2$[e]	−1.55	3.02	−0.42	0.27	−1.88	−0.27	0.981
		(0.13)	(0.09)	(0.09)	(0.11)	(0.05)	
$Cu(DS)_2$[e]	−1.51	3.05	−0.51	0.35	−1.92	−0.26	0.988
		(0.10)	(0.08)	(0.08)	(0.09)	(0.05)	

[a] Numbers in parentheses indicate the standard deviation for each coefficient. Separation conditions for the MEKC solutions are as follows.

[b] 40 m*M* surfactant; 10 m*M* sodium phosphate (pH 7); T=25°C; n=36.

[c] 40 m*M* SDS, 10 m*M* sodium phosphate (pH 7); T=35°C; n=35.

[d] 40 m*M* SDS, 10 m*M* sodium acetate (pH 4.5); T=35°C; n=35.

[e] 40 m*M* surfactant; no buffer (pH~4.5); T=35°C; n=35.

Table 3
Counter-ion effect on functional group selectivity[a]

Functional group	ΔΔ*G* (kJ/mol)					
	SDS	LiDS	SDS	SDS	$Mg(DS)_2$	$Cu(DS)_2$
(1) CH_3	−2.47	−2.33	−2.47	−2.45	−2.82	−2.58
	(0.02)	(0.05)	(0.03)	(0.15)	(0.06)	(0.05)
(2) CH_2CH_3	−4.68	−4.57	−4.73	−4.50	−5.48	−5.09
	(0.03)	(0.04)	(0.04)	(0.15)	(0.07)	(0.04)
(3) $CH_2CH_2CH_3$	−7.29	−7.20	−7.30	−7.17	−8.58	−7.99
	(0.05)	(0.04)	(0.03)	(0.17)	(0.11)	(0.13)
(4) CN	0.05	0.10	−0.26	−0.30	0.01	0.26
	(0.15)	(0.04)	(0.03)	(0.10)	(0.08)	(0.03)
(5) NO_2	−0.30	−0.31	−0.54	−0.50	−0.72	−0.20
	(0.15)	(0.05)	(0.02)	(0.12)	(0.44)	(0.06)
(6) O_2CCH_3	−1.02	−1.02	−1.20	−1.09	−0.88	−0.47
	(0.19)	(0.04)	(0.03)	(0.10)	(0.04)	(0.09)
(7) CO_2CH_3	−2.62	−2.83	−2.90	−2.95	−2.70	−2.46
	(0.04)	(0.04)	(0.02)	(0.10)	(0.06)	(0.10)
(8) OH	2.16	2.15	1.69	1.71	1.87	2.11
	(0.04)	(0.04)	(0.05)	(0.16)	(0.07)	(0.04)
(9) Cl	−2.88	−3.05	−3.10	−2.71	−3.35	−3.16
	(0.12)	(0.04)	(0.03)	(0.19)	(0.05)	(0.08)
(10) Br	−3.75	−3.86	−3.91	−3.63	−4.26	−4.01
	(0.12)	(0.07)	(0.02)	(0.14)	(0.07)	(0.08)
(11) I	−5.18	−5.30	−5.32	−4.87	−5.71	−5.48
	(0.02)	(0.08)	(0.02)	(0.19)	(0.06)	(0.13)

[a] Numbers in parentheses are the 95% confidence intervals for the ΔΔG values. All other superscript definitions are given in Table 2.

than dodecyl sulfate surfactants. We have found a similar trend in that the effect of the surfactant chain length is dependent on the nature of the head group [19]. More specifically, the chain length had a more significant influence on selectivity for the surfactants with a larger, less polar head group.

3.3. Comparison of divalent inorganic counterions to SDS

For the LSER analysis of the divalent species, different separation conditions were necessary. The divalent surfactant running buffers were prepared to be 40 m*M* surfactant. However, because of the extremely limited solubility of Mg^{2+} and Cu^{2+} at pH 7, these surfactant systems could not be buffered without the surfactant precipitating or introducing another cation to the solution. As a result, no buffer was used and the $Mg(DS)_2$ and $Cu(DS)_2$ solutions had pH values of 4.2 and 4.3, respectively. Therefore, a 40 m*M* SDS and 10 m*M* sodium acetate (pH 4.5) solution was also analyzed for a more complete comparison between monovalent and divalent counter-ion surfactants. In addition, the MEKC experiments for these systems were performed at 35°C in order to keep the divalent species from precipitating. Finally, it should also be noted that chloroaniline (solute 32 in Table 1) is positively charged at this pH which causes it to have strong electrostatic interactions with the anionic micelles. Therefore, it was omitted from the LSER analysis of these surfactants.

Table 2 also lists the LSER coefficients for these solutions. The results for SDS at pH 7.0 and 35°C are also listed in order to evaluate the effect of pH. Only one minor difference exists between these pH conditions. The *r* coefficient in Table 2 suggests that SDS at pH 4.5 has a slightly reduced capacity to interact with solute lone pair electrons at lower pH (e.g. less polarizable). Comparing SDS at pH 4.5 and 7.0, the less negative ΔΔ*G* values for the halogenated benzenes (solutes 9–11 in Table 3) for SDS at pH 4.5 confirm the LSER results. Otherwise, it is

readily apparent that there is little pH effect between pH 4.5 and 7.0. Even though the buffer composition and pH is different, the observed selectivity is nearly identical. It has been determined that the buffer type has very little influence on the LSER coefficients when comparing small buffer ions[2] (provided the same cation is used) [8,24]. Therefore, this similarity is unlikely to be the result of a coincidental canceling of effects from various interactions.

Comparing the divalent counter-ion species to one another shows that they have very similar selectivity behavior in MEKC. However, a rather significant difference is observed when comparing the two divalent species to SDS (pH 4.5). The LSER results in Table 2 show that $Mg(DS)_2$ and $Cu(DS)_2$ provide the solutes with a more 'hydrocarbon-like' microenvironment (more positive *m* coefficient), less polar (more negative *s*), and more polarizable (larger *r*) than SDS. The differences between *m* and *s* of the divalent surfactants and those of SDS is easily observed after looking at the change in free energy of transfer of non-hydrogen bonding substituents (solutes 1–3 in Table 3). This observation is consistent with the findings of Nielson and Foley [23]. They investigated $Mg(DS)_2$ micelles in the presence of various concentrations of acetonitrile and 4 m*M* EDTA. Under the MEKC conditions they used, $Mg(DS)_2$ proved to be a more hydrophobic micellar phase than SDS. These LSER results also show that $Mg(DS)_2$ and $Cu(DS)_2$ are also less likely to participate in hydrogen bonds than SDS. The more negative *b* and *a* coefficients show that in the presence of divalent counter-ions, dodecyl sulfate has weaker interactions with hydrogen bond accepting and donating phase, respectively. Again, the differences are statistically questionable, but the free energy of transfer data and other experimental results confirm these observations. Solutes 4–7 in Table 3 show that hydrogen bond accepting solutes have a less favorable free energy of transfer into the divalent surfactant systems. The same is observed for hydrogen bond donating solutes (solute 8 in Table 3).

[2]Other studies from this laboratory show that using large organic buffer additives [e.g. 3-cyclohexylamino-1-propanesulfonic acid (CAPS)] can have a significant influence on the observed selectivity in MEKC.

The individual influence of each type of solute–micelle interaction towards the free energy of transfer differences can be calculated by rearranging Eq. (4):

$$\begin{aligned}\Delta\Delta G &= -RT\ln\tau \\ &= -2.303RT\log[k'(\text{Bz-R})/k'(\text{Bz})]\end{aligned} \quad (4)$$

$$\Delta\Delta G = (-2.303RT)[\log k'(\text{Bz-R}) - \log k'(\text{Bz})] \quad (5)$$

Further rearrangement yields (Eq. (6)):

$$\begin{aligned}\Delta\Delta G = (-2.303RT)\{&m[V_x(\text{Bz-R}) - V_x(\text{Bz})] \\ &+ s[\pi^*_2(\text{Bz-R}) - \pi^*_2(\text{Bz})] \\ &+ r[R_2(\text{Bz-R}) - R_2(\text{Bz})] \\ &+ b[\Sigma\beta_2(\text{Bz-R}) - \Sigma\beta_2(\text{Bz})] \\ &+ a[\Sigma\alpha_2(\text{Bz-R}) - \Sigma\alpha_2(Bz)]\} \\ = (-2.303RT)[&m\Delta(V_x) + s\Delta(\pi^*_2) + r\Delta(R_2) \\ &+ b\Delta(\Sigma\beta_2) + a\Delta(\Sigma\alpha_2)]\end{aligned} \quad (6)$$

Therefore, by using the LSER coefficients and the changes in solute parameters (relative to benzene), it is possible to estimate the contribution of each individual source of solute–micelle interaction for different functional groups. Although the difference between the $\Delta\Delta G$ values calculated using Eq. (4) and Eq. (6) is large for a few solutes, the agreement is generally good. The solutes that have poor agreement are typically early eluters near t_{eo} where accurate estimation of k′ is difficult. However, meaningful qualitative information can still be obtained despite of this.

Tables 4 and 5 show the results for SDS (pH 4.5) and $Cu(DS)_2$. Similar to Table 3, the more negative (or less positive) the $\Delta\Delta G$ value, the more favorable the interaction. A general observation is that the solute size and lone pair electrons all favor retention in MEKC, and the polarity and hydrogen bonding characteristics of the solutes impede their retention in these pseudo-stationary phases. Separating the contributions also shows that the most influential component of retention differences between solutes in a given surfactant system is solute size. Rather surprisingly, this is true even for hydrogen bond accepting solutes. The largest influence on phenol retention (No. 8 in Tables 4 and 5) is its hydrogen bond accepting ability. Its polarity also has larger relative

Table 4
Individual contributions to $\Delta\Delta G$ for SDS (pH 4.5)

Functional group	$\Delta\Delta G$ (kJ/mol)				
	$m\Delta(V_x)^*$ $-2.303RT$	$s\Delta(\pi^*_2)^*$ $-2.303RT$	$r\Delta(R_2)^*$ $-2.303RT$	$b\Delta(\Sigma\beta_2)^*$ $-2.303RT$	$a\Delta(\Sigma\alpha_2)^*$ $-2.303RT$
(1) CH_3	−2.41	0	0.01	0	0
(2) CH_2CH_3	−4.82	−0.02	0	0.10	0
(3) $CH_2CH_2CH_3$	−7.22	−0.03	0.01	0.10	0
(4) CN	−2.65	0.91	−0.13	1.91	0
(5) NO_2	−2.99	0.91	−0.26	1.40	0
(6) O_2CCH_3	−6.10	0.94	−0.05	4.01	0
(7) CO_2CH_3	−6.10	0.51	−0.12	3.21	0
(8) OH	−1.00	0.57	−0.20	1.61	0.65
(9) Cl	−2.10	0.20	−0.11	−0.70	0
(10) Br	−2.99	0.33	−0.27	−0.50	0
(11) I	−4.42	0.46	−0.58	−0.20	0

influence than other solutes, and its size contribution is markedly less important. The influence of phenol's hydrogen bond donating ability is dependent on the surfactant system.

The retention of halogenated solutes also has some notable trends. As the electronegativity of the halogens increases, the hydrogen bond accepting ability of the halogenated benzene decreases (Table 1). Chlorobenzene (No. 9 in Tables 4 and 5) is consistently retained longer than toluene (No. 1) in every surfactant in this report. The results in Tables 4 and 5 show that the primary reason for this is chlorobenzene decreased capacity as a hydrogen bond acceptor. Furthermore, as the halogen size increases (and consequently its electronegativity decreases), the hydrogen bonding properties become less important, and the lone pair electron contribution [$r\Delta(R_2)$] to retention becomes more important.

The elution patterns for a test mixture of uncharged solutes in SDS (pH 4.5 and 7.0), $Mg(DS)_2$, and $Cu(DS)_2$ are presented in Figs. 1–4. The solutes used included two nonhydrogen bonding (toluene and propylbenzene), two hydrogen bond acceptors (methyl 2-methylbenzoate and ethylbenzoate), and one hydrogen bond donor (*p*-bromophenol). In Fig. 1 it is clear that baseline separation is achieved for all of the solutes in SDS (pH 4.5). In addition, the hydrogen bond accepting solutes are the longest

Table 5
Individual contributions to $\Delta\Delta G$ for $Cu(DS)_2$

Functional group	$\Delta\Delta G$ (kJ/mol)				
	$m\Delta(V_x)^*$ $-2.303RT$	$s\Delta(\pi^*_2)^*$ $-2.303RT$	$r\Delta(R_2)^*$ $-2.303RT$	$b\Delta(\Sigma\beta_2)^*$ $-2.303RT$	$a\Delta(\Sigma\alpha_2)^*$ $-2.303RT$
(1) CH_3	−2.53	0	0.02	0	0
(2) CH_2CH_3	−5.07	−0.03	−0.01	0.11	0
(3) $CH_2CH_2CH_3$	−7.60	−0.06	0.01	0.11	0
(4) CN	−2.79	1.79	−0.27	2.16	0
(5) NO_2	−3.14	1.79	0.54	1.59	0
(6) O_2CCH_3	−6.42	1.85	−0.11	4.54	0
(7) CO_2CH_3	−6.42	1.00	−0.25	3.63	0
(8) OH	−1.06	1.12	−0.40	1.82	0.91
(9) Cl	−2.21	0.39	−0.22	−0.79	0
(10) Br	−3.14	0.64	−0.56	−0.57	0
(11) I	−4.65	0.91	−1.19	−0.23	0

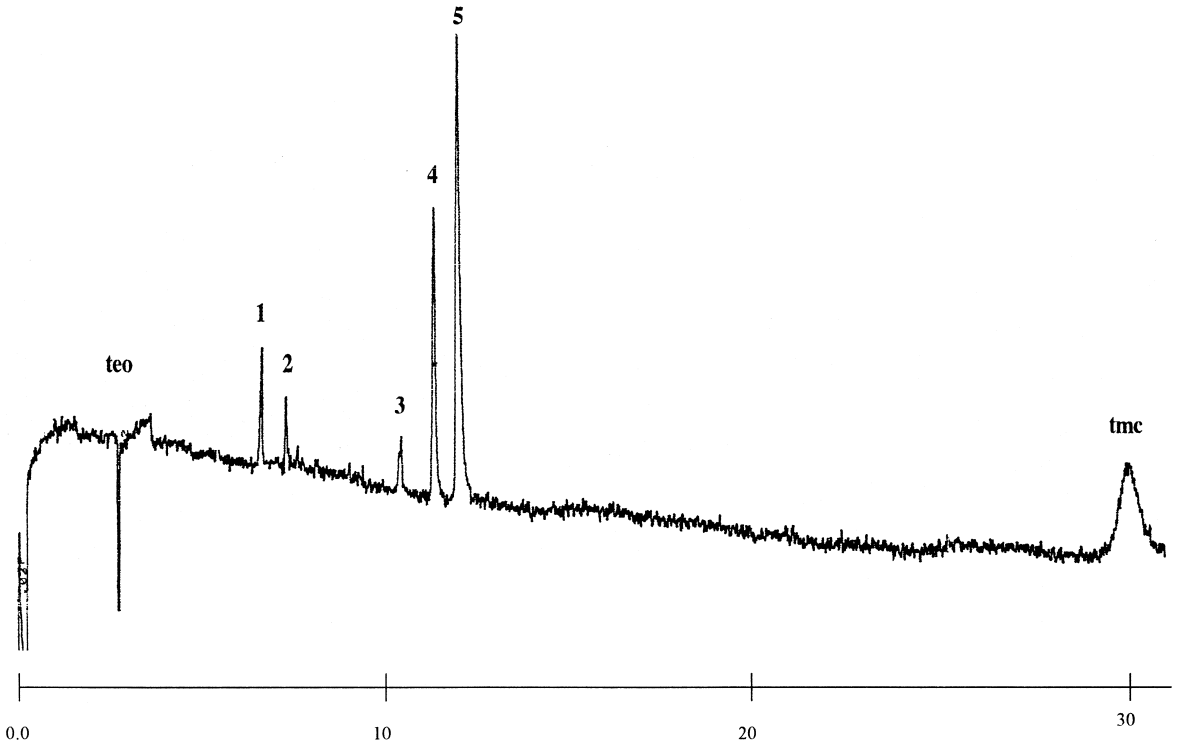

Fig. 1. Electropherogram for a set of test solutes in 40 m*M* SDS, 10 m*M* sodium acetate buffer (pH 4.5). Experimental conditions as described in Experimental. 1=Toluene, 2=*p*-bromophenol, 3=propylbenzene, 4=methyl-2-methylbenzoate, 5=ethylbenzoate. Time scale in min.

retained in this system. The same elution order and selectivity is observed for SDS buffered at pH 7 (Fig. 2). However, the lower pH significantly reduces the EOF flow and makes the elution window much longer ($t_{mc}/t_{eo} = 11.13$). Although a larger retention window is often desirable in MEKC, in this case it is at the expense of efficiency. The efficiency for the SDS (pH 4.5) system is approximately three times less that of the $Mg(DS)_2$ and $Cu(DS)_2$ systems ($N_{Na} \sim 50\,000$).

The elution window for the divalent dodecyl sulfate surfactants are significantly smaller than that of SDS, but efficiencies are higher and elution pattern variations are still observed. Fig. 3 shows that $Mg(DS)_2$ has the smallest elution window ($t_{mc}/t_{eo} = 2.28$) and the highest efficiency ($N_{Mg} \sim 180\,000$). In addition, an elution order reversal is observed between the bromophenol–toluene pair as well as between methyl 2-methylbenzoate and propylbenzene. Fig. 4 shows that $Cu(DS)_2$ has a moderate elution window ($t_{mc}/t_{eo} = 2.98$) and efficiency ($N_{Cu} \sim 150\,000$). Compared to the elution order in SDS, Fig. 4 also shows that using copper as the counter-ion significantly reduces the surfactant participation in hydrogen bonding interactions with solutes.

The retention of all of the solutes is larger in $Mg(DS)_2$ and $Cu(DS)_2$ than SDS. This is the result of a higher phase ratio for these systems at the same molar concentration as SDS. This is also apparent in the LSER model constant, *c*, in Table 2. The less negative value for *c* obtained for the divalent counter-ion surfactants suggests that their phase ratio is higher. However, although the phase ratio does influence the solute retention factor, it does not affect the observed selectivity [3]. Using the electropherograms in Figs. 1–4, the retention factors of five solutes in SDS, $Mg(DS)_2$, and $Cu(DS)_2$ as well as the k' ratio between the divalent species versus the SDS are listed in Table 6. The increase in k' for

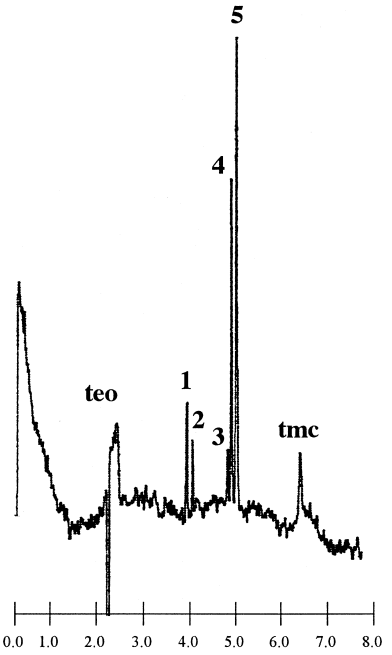

Fig. 2. Elution order for test mixture in 40 m*M* SDS, 10 m*M* sodium phosphate buffer (pH 7.0). Experimental conditions and solute identification are given in Fig. 1. Time scale in min.

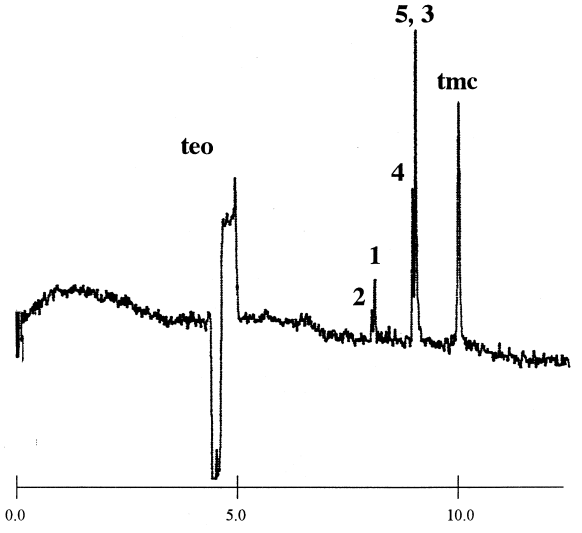

Fig. 3. Elution order for test mixture in 40 m*M* $Mg(DS)_2$ in the absence of buffer (pH 4.2). Experimental conditions and solute identification are given in Fig. 1. Time scale in min.

hydrogen bond accepting solutes is not as large as the increase for non-hydrogen bonding solutes because the divalent counter-ion species form weaker hydrogen bond donating phases. This shows that although the phase ratio influences the solute retention factor, the increase in retention is dependent upon structural properties of the solute.

3.4. Source of selectivity differences

A two-state model where a solute can interact with micelles by being either partitioning into the micelle interior or adsorbed onto the micelle surface has been discussed in the literature [25]. Based on this model, most of the solutes used in this analysis possess a fairly high surface activity meaning that the environment in the outer region of the micelles primarily determines the solubilization. As has been discussed previously, it is believed that the observed polarity and hydrogen bond donating characteristics of micellar phases are determined by the amount of water in the micelle palisade and Stern layers [17].

With this in mind, it is interesting to compare the physicochemical properties of dodecyl sulfate surfactants in the presence of various counter-ions. First, the micelles of $Mg(DS)_2$ and $Cu(DS)_2$ have a significantly larger radius and aggregation number than SDS [26]. Almgren and Swamp then showed that the micelle size does not increase proportionally with the aggregation number when going from monovalent to divalent surfactant counter-ions [27]. In that report, they determined that the number of surfactant monomers per micelle area ($Å^2$) increased when divalent cations were introduced to the micellar solution. Examples of these results are listed in Table 7. Almgren and Swamp also found that the value of $N_{agg}/Å^2$ is only dependent on the valency of the counter-ion and not the type of cation. Therefore, although the $Cu(DS)_2$ micellar size is not available, it is reasonable to believe that it follows the same trend. Using Almgren and Swamp's data, it can be seen that the average micellar surface area occupied by a surfactant monomer decreases for divalent counter-ions relative to SDS (Table 7). This suggests that the dodecyl sulfate chains are more densely packed for divalent species, and therefore, form aggregates that do not allow as much water to

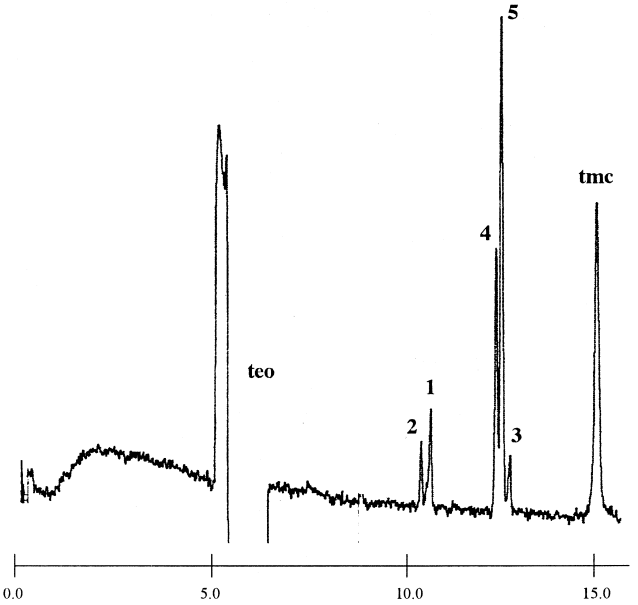

Fig. 4. Elution order for test mixture in 40 m*M* $Cu(DS)_2$ in the absence of buffer (pH 4.3). Experimental conditions and solute identification are given in Fig. 1. Time scale in min.

Table 6
Effect of counterion on increased retention in MEKC

Solute	k'			$[k'X(DS)_2]/[k'SDS]$	
	SDS (pH 4.5)	$Mg(DS)_2$	$Cu(DS)_2$	$Mg(DS)_2$	$Cu(DS)_2$
Toluene	1.83	4.52	3.90	2.47	2.13
4-Bromophenol	2.20	4.24	3.52	1.93	1.60
Propylbenzene	4.33	11.26	10.24	2.60	2.36
Methyl-2-methylbenzoate	5.03	10.30	8.52	2.05	1.69
Ethybenzoate	5.57	11.26	9.17	2.02	1.65

penetrate the micelle surface and reside in the palisade layer. This reduction in the amount of water present is responsible for the weaker hydrogen bond donating and less polar phases for divalent counter-ion surfactants. By comparing SDS with and without the presence of Mg^{2+} counter-ions in solutions, Huang and Bright have found the same trend spectroscopically using two different fluorescent probes to study different regions of the micelle aggregates [28].

Table 7
Micelle aggregation number (N_{agg}) radius (R) and area per headgroup for various dodecyl sulfate surfactants[a]

Counterion	N_{agg}	R (Å)	$Å^2$/monomer
Na^+	68	17.9	59.5
Mg^{2+}	96	20.1	53.2
Cd^{2+}	87	19.5	54.9
Cu^{2+}	95 [26]		

[a] Aggregation number and micelle radius data was obtained from Ref. [27] unless otherwise noted.

It is not the intention of this paper to critically evaluate the effect of temperature on selectivity in MEKC. In fact, studies are currently being done in this laboratory to investigate these effects in more detail [29]. However, we would like to note that temperature might influence selectivity as well as retention in MEKC. As can be seen in Tables 2 and 3, the LSER model predicts that increasing the temperature by 10°C results in slightly increased

retention of polar/polarizable and hydrogen bond accepting solutes (less negative *b* and larger *r* coefficients, respectively).

4. Conclusions

Surfactant counter-ions do have a modest influence on selectivity in MEKC. The effect seems to be dependent only on the valency of the counter-ion and not the type of counter-ion present. It is believed that observed effects are a result of the divalent counter-ions effectively reducing the electrostatic repulsion between adjacent surfactant head groups in micelle aggregates. As a result, less water is allowed to penetrate beyond the micelle surface making the environment observed by solutes less polar and less hydrogen bond donating. In addition, comparing these results with those from previous reports suggests that the degree to which the counter-ion influences selectivity may vary for surfactants with different head groups [21].

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