

Quantitative structure–activity relationships studies with micellar electrokinetic chromatography

Influence of surfactant type and mixed micelles on estimation of hydrophobicity and bioavailability

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Abstract

Applications of micellar electrokinetic chromatography (MEKC) in quantitative structure–activity relationships (QSAR) were studied. First, quantitative structure–retention relationships (QSRR), which describe the correlation between logarithm of capacity factor ($\log k'$) in MEKC and logarithm of distribution coefficient between 1-octanol and water ($\log P_{ow}$), were investigated for 60 aromatic compounds and 9 corticosteroids using three different anionic surfactants [e.g., sodium dodecyl sulfate (SDS), sodium cholate (SC), and lithium perfluorooctane sulfonate (LiPFOS)], one cationic surfactant (C_{14} TAB), and mixed anionic micellar systems. Linear solvation energy relationships (LSER) and solvatochromic parameters were used to shed light on the different $\log k'$ vs. $\log P_{ow}$ relationships for the various surfactants. It was concluded that hydrogen bonding interactions have a great influence on retention behavior in MEKC and its relationship with hydrophobicity. Interestingly, bile salt surfactants (e.g., SC) and mixed bile salt micellar systems provide better correlations for $\log k'$ vs. $\log P_{ow}$ than SDS and/or SDS with buffer additives (e.g., β -cyclodextrin, urea, and acetonitrile). Using SC micelles, only one line was adequate to describe the relationship between retention in MEKC and hydrophobicity for a group of 60 aromatic compounds. The existence of higher correlation for the SC system was attributed to a similar hydrogen bonding pattern between SC micelles and 1-octanol. In the SDS system, however, three lines were recognized for the congeneric subgroups of compounds. This is due to the hydrogen bond donor (HBD) characteristic of SDS micelles that selectively differentiate between the solutes with different hydrogen bond acceptor (HBA) strength, thus demonstrating that retention is not solely based on hydrophobicity. A similar result was observed for a C_{14} TAB-MEKC system, however, the HBA characteristic of C_{14} TAB selectively differentiates between the solutes with different HBD strength.

In addition, quantitative retention–activity relationships in MEKC were also investigated for 9 corticosteroids. Two types of biological activities [small intestinal absorption in the rat ($\log A/NA$) and protein binding to human serum albumin ($\log B/F$)] were examined in this work. High correlations were observed between bioactivity and $\log k'$ in MEKC using bile salt surfactants and mixed bile salt systems.

Keywords: Micellar electrokinetic chromatography; Quantitative structure–activity relationships; Hydrophobicity; Partition coefficients; Micelles; Protein binding; Surfactants; Sodium dodecyl sulfate; Bile salts; Tetradecyltrimethylammonium bromide

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1. Introduction

In quantitative structure–activity relationships (QSAR), biological activities of solutes are related to their physico-chemical and/or structural descriptors such as distribution coefficients between 1-octanol and water. Hansch and co-workers introduced the modern area of QSAR by showing linear relationships between biological response and distribution coefficients between 1-octanol and water, P_{ow} as:

$$\log(1/C) = a \log P_{ow} + b \quad (1)$$

where C is the concentration of bioactive compounds that induces a certain activity [1,2].

Potency and availability of many bioactive compounds, bioaccumulation of organic molecules in organic biota, and sorption of environmental pollutants on organic-rich soils have been correlated to their lipophilicity. Over the past three decades, due to its predictive and diagnostic nature, the modern field of QSAR has become a significant part of various disciplines such as drug design, toxicology, and environmental monitoring.

Eq. 1 is an example of linear free energy relationships (LFER). According to LFER, linear relationships exist between changes in free energies of physico-chemical processes with similar mechanisms or interactions. Since hydrophobic interactions also play a major role in chromatographic retention in systems such as reversed-phase LC (RPLC) and micellar electrokinetic chromatography (MEKC), one can expect linear relationships between capacity factor and $\log P_{ow}$ as:

$$\log k' = a \log P_{ow} + b \quad (2a)$$

or

$$\log P_{ow} = a' \log k' + b' \quad (2b)$$

where $\log k'$ is the logarithm of capacity factor (also referred to as retention factor or migration factor) in RPLC or MEKC and $\log P_{ow}$ is the logarithm of distribution coefficients of solutes between 1-octanol and water [1,2]. These equations are examples of quantitative structure–retention relationships (QSRR).

During the past three decades, $\log P_{ow}$ has become the accepted quantitative scale for lipophilicity of molecules [1–3]. However, determination of $\log P_{ow}$ is still a difficult task. The direct measurement of $\log P_{ow}$, using the conventional shake-flask (SF) method, is time consuming, tedious, requires highly pure compounds in reasonable quantities, and has a limited dynamic range. An alternative is the use of RPLC for indirect determination of $\log P_{ow}$ through linear correlation with the logarithm of capacity factor according to Eq. 2b [3].

This indirect method has received much attention due to the unique advantages of HPLC such as small sample size, speed, high sample throughput, better reproducibility, suitability for substances containing impurities and mixtures, wider dynamic range, and feasibility for automation [3–6]. The use of RPLC for estimation of $\log P_{ow}$ suffers from several shortcomings [3–6]. First, capacity factor in HPLC is not a continuous scale and is a function of mobile- and stationary-phase compositions. In addition, various relationships are observed for different groups of congeneric compounds. Also, determination of $\log P_{ow}$ values for ionizable compounds is difficult.

Micellar electrokinetic chromatography (MEKC) [7–9] has several potential advantages in structure–activity studies. It is noteworthy that MEKC offers all of the above mentioned advantages for physico-chemical characterization of molecules [7–9]. First, MEKC offers the same features for physico-chemical measurements as HPLC (i.e., no sample purity requirement, small sample size, automation, etc.). Second, it is feasible to adjust the composition of the micellar pseudo-stationary phase by simply changing the type of surfactant(s) in the system in order to provide better chemical models for the interactions in biological systems, or for facilitating $\log P_{ow}$ determination. Third, micelles are amphiphilic aggregates with anisotropic microenvironments that provide both hydrophobic and electrostatic sites of interactions. In this respect they are more structurally similar to biomembranes than 1-octanol or RPLC stationary phases (the latter is anisotropic but not amphiphilic).

[10,11]. Fourth, a great potential in MEKC is the possibility of standardization of retention. Note that in MEKC, retention factor is directly related to the distribution coefficients into micelles which by definition is a single and continuous scale for a given surfactant system. An advantage of $\log P_{ow}$ is that it is a single and continuous scale, at least in theory; although in practice there exists large variations in the P_{ow} values (by as much as one order of magnitude) which reflects the difficulties of the measurements.

There have been several reports involving the relationships between water–micelle distribution coefficients, $\log P_{mw}$, with anesthetic action [12] as well as $\log P_{ow}$ [13–15]. For example, Marina et al. [13], Valsaraj and Thibodeaux [14], and Treiner [15] have previously reported the relationships between micelle–water partition coefficients, $\log P_{mw}$, and $\log P_{ow}$. A main obstacle in using micelles in QSAR studies has been the difficulty of measuring P_{mw} by the classical methods. This problem can be easily solved by MEKC as capacity factor is directly related to the water–micelle distribution coefficient as [7–11]:

$$k' = P_{mw} \nu (C_{sf} - CMC) \quad (3)$$

where P_{mw} is the partition coefficient of a solute between the micellar pseudophase and the aqueous phase, ν is the molar volume of surfactant, C_{sf} is the surfactant concentration, and CMC is the critical micelle concentration.

The relationships between $\log k'$ in MEKC and $\log P_{ow}$ have been reported in at least four publications [16–19]. In all studies sodium dodecyl sulfate (SDS) was used. In one case [17], mixed SDS–Brij35 and CTAB surfactants have been examined for a limited group of compounds. Since the first introduction of MEKC, SDS has been the most widely used surfactant. However, for the estimation of $\log P_{ow}$ of certain groups of compounds, SDS might not be the most suitable system. In fact, as will be shown in the following sections, congenerity problems exist in the SDS–MEKC system; that is, different $\log k'$ vs. $\log P_{ow}$ relationships are observed for various groups of compounds. As

mentioned above, other workers have also reported congeneric problems for $\log P_{mw}$ vs. $\log P_{ow}$ [12–15].

In MEKC, retention behavior of solutes depends strongly on the type of surfactant. It is not surprising that the relationships between $\log k'$ and $\log P_{ow}$ for the same group of compounds can be greatly different between various micellar systems. The variations in $\log P_{ow}/\log k'$ relationships are indicative of chemical selectivity of different surfactants. This suggests that hydrophobic interaction is not the only underlying force that influences retention in MEKC.

We have previously reported the use of micellar liquid chromatography (MLC) in QSAR studies [10,11]. In this work, the applications of MEKC for quantitation of lipophilic character of molecules and estimation of bioactivity are investigated using different types of surfactant.

2. Experimental

All experiments were carried out on laboratory-built CE systems that consisted of 0–30 kV high-voltage power supplies (Series EH, Glassman High Voltage, Whitehouse Station, NJ, USA) and 50 μm I.D., 375 μm O.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The total length of the capillaries was 62 cm and detection was performed 50 cm downstream. Two variable-wavelength UV detectors (Model 200, Linear Instruments, Reno, NV, USA; and Model 500, Scientific Systems, Inc. (SSI), State College, PA, USA) were used in this work. The wavelengths of 210 nm for sodium dodecyl sulfate (SDS) buffers, 214 nm for lithium perfluorooctane sulfonate (LiPFOS), and 254 nm for all other buffers were used. Two integrators (Model SP 4200, Spectra Physics, San Jose, CA, USA; and Hewlett-Packard, Avondale, PA, USA) were used to record the electropherograms. The samples were introduced into the anodic end of the capillary by gravity, 10 cm height for 8 s. A positive voltage of 20 kV was applied for the group of 60 aromatic compounds with SDS, SC, and LiPFOS, while a positive voltage of 15 kV was applied with the steroids. A

voltage of -20 kV was used with C_{14} TAB because the electroosmotic flow was reversed.

2.1. Reagents

All 60 aromatic compounds (as shown in Table 1) and urea were purchased from Aldrich (Milwaukee, WI, USA). Steroids (also in Table 1) were purchased from Sigma (St. Louis, MO, USA) and Steraloids (Wilton, NH, USA). Sodium dodecyl sulfate (SDS, 99% pure), tetracycltrimethylammonium bromide (C_{14} TAB), and β -cyclodextrin (β -CD) were purchased from Sigma. Bile salts were purchased from Aldrich and Sigma. Lithium perfluorooctane sulfonate (LiPFOS) was a gift from 3M Corp. (St. Paul, MN, USA). Acetonitrile was purchased from Fisher Scientific (Raleigh, NC, USA). All buffer solutions were prepared by dissolving appropriate amounts of surfactant in doubly distilled deionized water and were filtered through a $0.45\text{-}\mu\text{m}$ nylon-66 membrane filter (Rainin, Woburn, MA, USA). Buffer solutions for the group of 60 aromatic compounds were kept at pH 7.0 and 0.050 M phosphate (ionic strength), while buffers for the steroids were kept at pH 9.0 and 0.050 M phosphate/borate buffer. Room temperature (25°C) was maintained throughout the experiments. The migration time of an unretained solute (i.e., t_{e0}) was measured from the time of injection to first deviation of the baseline using methanol as the marker. Micellar migration time, t_{mc} , was measured using n -dodecanophenone as the marker. With all four surfactants, the run times were between 10 and 15 min, for instance, it was about 10 min with 80 mM SC; and about 13 min with 40 mM SDS [20].

3. Results and discussion

3.1. Effect of surfactant type on $\log k'$ vs. $\log P_{ow}$ relationships

The results of $\log k'$ vs. $\log P_{ow}$ linear regression are listed in Table 2 for a group of 60 aromatic compounds. The correlation coeffi-

cients for SDS, C_{14} TAB and SC micellar systems are high; however, a poor correlation was observed for the LiPFOS system. There exist significant differences between 1-octanol and LiPFOS micelles, while the retention in SC-MEKC systems highly correlates with $\log P_{ow}$ (Figs. 2 and 3). Figs. 1–4 provide more details about the exact nature of these relationships.

Since the first introduction of MEKC in 1984, it has been generally accepted that retention in MEKC is according to solutes' hydrophobicity. The large differences in retention behavior in MEKC with different types of surfactants (Figs. 1–4) indicate, however, that this general belief is not accurate for all MEKC systems.

Recently, we reported the use of linear solvation energy relationships (LSER) and functional group selectivity data for characterization of solute interactions with three types of micelles: sodium dodecyl sulfate (SDS), sodium cholate (SC) and lithium perfluorooctane sulfonate (LiPFOS) [20,21]. The LSER results shed light on the selectivity differences between the four micellar systems and can be quite useful in identifying MEKC systems that are most suitable for estimation of $\log P_{ow}$.

According to our LSER results the pattern of chemical interactions in the SC micellar system among three micellar systems is closer to that in 1-octanol–water system, while, the LiPFOS micelles has the largest difference with 1-octanol–water. The LSER analysis indicates that the SC micelles and 1-octanol have similar hydrogen bond acceptor characteristic, while LiPFOS micelles have a strong hydrogen bond donor capability; SDS is a somewhat weaker hydrogen bond donor than LiPFOS [20,21]. It was also concluded that selectivity differences between these surfactant types in MEKC are primarily due to hydrogen bonding interactions, rather than dipolar interactions. Consequently, retention of solutes in MEKC would correlate best with $\log P_{ow}$ and exhibit the least congeneric behavior if a micellar pseudo-stationary phase is used that has a similar hydrogen bonding affinity as 1-octanol.

The LiPFOS micelles are the most cohesive organic phase, the strongest hydrogen bond donors and the weakest hydrogen bond accep-

Table 1
Test solutes and their hydrophobicity (bioactivity) in MEKC

Compound	$\log P_{ow}^a$	Compound	$\log P_{ow}^a$
1. Benzene	2.13	31. Phenyl acetate	1.49
2. Toluene	2.69	32. Phenol	1.49
3. Ethyl benzene	3.15	33. 4-Methylphenol	1.94
4. Propyl benzene	3.68	34. 4-Ethylphenol	2.58
5. <i>p</i> -Xylene	3.15	35. 4-Fluorophenol	1.77
6. Acetophenone	1.58	36. 4-Chlorophenol	2.35
7. Propiophenone	2.19	37. 4-Bromophenol	2.59
8. Butyrophenone	2.76	38. 4-Iodophenol	2.91
9. Valerophenone	3.56	39. Benzyl alcohol	1.08
10. Benzylaldehyde	1.48	40. 4-Methylbenzyl alcohol	1.59
11. Benzonitrile	1.56	41. 4-Chlorobenzyl alcohol	1.96
12. Nitrobenzene	1.85	42. Aniline	0.90
13. Anisole	2.11	43. <i>N</i> -Ethylaniline	2.16
14. Ethoxybenzene	2.51	44. 4-Chloroaniline	1.83
15. Methyl benzoate	2.16	45. 4-Bromoaniline	2.05
16. Ethyl benzoate	2.64	46. Pyridine	0.65
17. Fluorobenzene	2.27	47. Naphthalene	3.35
18. Chlorobenzene	2.84	48. 1-Methyl naphthalene	3.87
19. Bromobenzene	2.99	49. 2-Methyl naphthalene	3.86
20. Iodobenzene	3.25	50. Anthracene	4.54
21. <i>p</i> -Dichlorobenzene	3.38	51. Biphenyl	3.90
22. <i>o</i> -Dichlorobenzene	3.28	52. 3-Chlorophenol	2.49
23. 2-Chloronitrobenzene	2.24	53. 3-Methylphenol	1.96
24. 4-Chloronitrobenzene	2.41	54. 2-Methylphenol	1.95
25. 4-Chlorotoluene	3.33	55. 3-Bromophenol	2.63
26. 4-Chloroanisole	2.82	56. 3-Methylbenzyl alcohol	1.58
27. 1-Bromo-4-nitrobenzene	2.55	57. 3-Chlorobenzyl alcohol	1.94
28. 4-Nitrotoluene	2.45	58. Phenethyl alcohol	1.36
29. 4-Chloroacetophenone	2.35	59. 3-Phenyl-1-propanol	1.88
30. Methyl-2-methylbenzoate	2.75	60. 3,5-Dimethylphenol	2.35
Steroids	$\log P_{ow}^b$	$\log (A/NA)^c$	$\log (B/F)^d$
Cortisone	1.42	-0.27	0.23
Prednisone	1.46	-0.74	0.16
Deoxycorticosterone	2.90	0.71	0.95
Prednisolone	1.62	-0.70	0.10
Corticosterone	1.94	-0.06	0.66
Hydrocortisone	1.55	-0.58	0.25
Progesterone	3.87	1.19	1.19
Triamcinolone	1.03	-0.89	-0.12
6 α -Methylprednisolone	1.85	- ^c	0.55

^a $\log P_{ow}$ values from Ref. [1].

^b Values from Ref. [25].

^c Values from Ref. [26].

^d Values from Ref. [25].

^e Absorption activity value for 6 α -methyl prednisolone is not available in Ref. [25].

Table 2

Quantitative relationships between hydrophobicity and migration factors in MEKC

	$\log k' = a \log P_{ow} + b$				
	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	S.E.
0.020 M SDS	0.60 (0.01) ^a	-1.28	60	0.9259	0.194
0.040 M SDS	0.60 (0.01) ^a	-0.95	60	0.9301	0.189
0.060 M SC	0.69 (0.00) ^a	-1.61	60	0.9806	0.110
0.080 M SC	0.69 (0.00) ^a	-1.46	60	0.9829	0.103
0.010 M C ₁₄ TAB	0.60 (0.01) ^a	-1.31	60	0.8877	0.248
0.040 M LiPFOS	0.30 (0.02) ^a	-0.86	60	0.5595	0.352

^a 95% confidence interval.*n* = number of test solutes; *r* = correlation coefficient of linear regression; S.E. = standard error of $\log k'$ estimated. Sodium dodecyl sulfate (SDS), sodium cholate (SC), lithium perfluorooctane sulfonate (LiPFOS).

tors. On the other hand, 1-octanol has the least cohesive character among the four systems, is the weakest hydrogen bond donor, and the strongest hydrogen bond acceptor. Therefore, it is not surprising that the overall trend in MEKC retention behavior with LiPFOS micelles does not correlate with the $\log P_{ow}$ scale.

For the SDS-MEKC system, the existence of three $\log k'$ vs. $\log P_{ow}$ lines for congeneric

solutes is notable (see Fig. 1). This is an indication of the selective interactions with SDS micelles that lead to different retention behavior of various groups of solutes. Since SDS micelles are stronger hydrogen bond donors than 1-octanol, they exhibit more selective interactions towards hydrogen bond acceptor solutes. In fact, one can recognize a trend in the grouping of various solutes in the three lines according to their

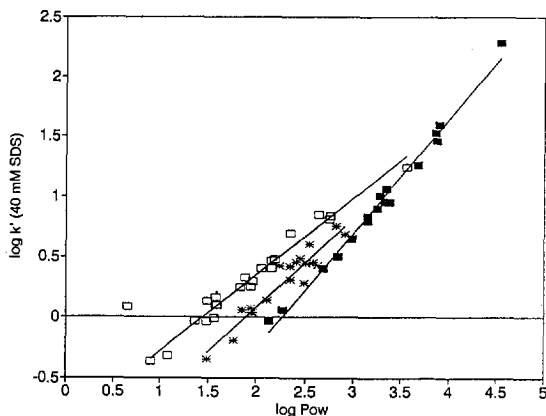


Fig. 1. Relationships between $\log k'$ and $\log P_{ow}$ for MEKC systems with 0.040 M SDS for a group of 60 aromatic compounds. $\log k' = (0.60 \pm 0.01)\log P_{ow} - 0.95$; $n = 60$, $r = 0.9301$, S.E. = 0.189.

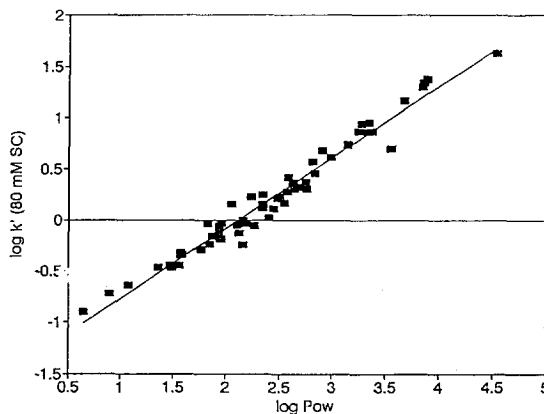


Fig. 2. Relationships between $\log k'$ and $\log P_{ow}$ for MEKC systems with 0.080 M SC for a group of 60 aromatic compounds. $\log k' = (0.69 \pm 0.00)\log P_{ow} - 1.46$; $n = 60$, $r = 0.9829$, S.E. = 0.103.

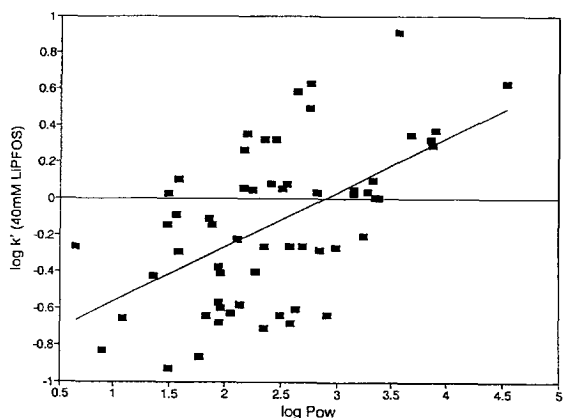


Fig. 3. Relationships between $\log k'$ and $\log P_{ow}$ for MEKC systems with 0.040 M LiPFOS for a group of 60 aromatic compounds. $\log k' = (0.30 \pm 0.02)\log P_{ow} - 0.86$; $n = 60$, $r = 0.5595$, S.E. = 0.352.

hydrogen bond acceptor (HBA) strength (as represented by Kamlet and Taft solvatochromic β -values) [20]. For example, subgroup A (bottom line with filled squares) consists of weak hydrogen bond acceptors ($\beta \leq 0.2$) which are hydrophobic substituted aromatic compounds such as alkylbenzenes, halogenated benzenes, and polycyclic aromatic hydrocarbons (PAHs). We referred to this group as nonhydrogen bonding (NHB) solutes previously [20]. For this subgroup A:

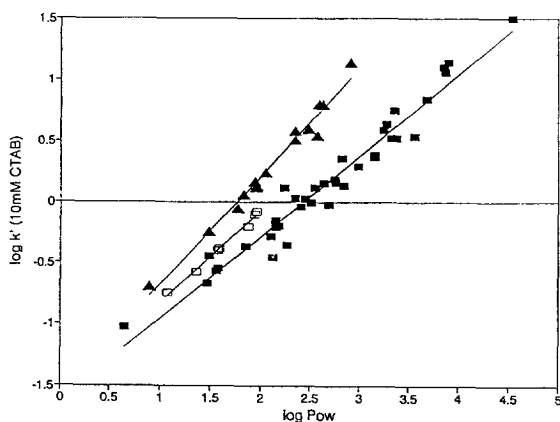


Fig. 4. Relationships between $\log k'$ and $\log P_{ow}$ for MEKC system with 0.010 M C14TAB for a group of 60 aromatic compounds. $\log k' = (0.60 \pm 0.01)\log P_{ow} - 1.31$; $n = 60$, $r = 0.8877$, S.E. = 0.248.

$$\log k'(40\text{mM SDS}) = (0.98 \pm 0.01) \log P_{ow} - 2.15$$

$$n = 17, r = 0.9940, \text{S.E.} = 0.065 \quad (4)$$

The second subgroup, B (middle line with asterisks), contains HBA compounds with intermediate strength ($0.2 < \beta < 0.35$) such as aromatic ethers, and substituted nitrobenzenes. Note that some phenolic compounds that have both HBA and hydrogen bond donor (HBD) characteristics are also in this group. For this subgroup B:

$$\log k'(40\text{mM SDS}) = (0.74 \pm 0.03) \log P_{ow} - 1.39$$

$$n = 20, r = 0.9551, \text{S.E.} = 0.088 \quad (5)$$

The third subgroup, C (upper line with open squares), includes the strongest HBA among all solutes ($\beta > 0.35$) such as alkyl aromatic ketones (acetophenone, propiophenone, butyrophenone, and valerophenone), benzonitrile, aromatic esters, and anilines. This group also contains solutes with HBA and HBD characters like aromatic alcohols. For subgroup C:

$$\log k'(40\text{mM SDS}) = (0.62 \pm 0.01) \log P_{ow} - 0.91$$

$$n = 22, r = 0.9877, \text{S.E.} = 0.063 \quad (6)$$

A similar behavior in the $\log k'$ vs. $\log P_{ow}$ was observed in the C_{14} TAB-MEKC system (Fig. 4). Three different lines are observed in the plot mainly due to the differences in solutes' hydrogen bonding donor acidity (as represented by Kamlet and Taft α -values) because C_{14} TAB provides more basic environment than 1-octanol. For example, subgroup A (bottom line with filled squares) consists of weak hydrogen bond donors ($\alpha \leq 0.17$) which include hydrophobic substituted aromatic compounds such as alkylbenzenes, halogenated benzenes, and polycyclic aromatic hydrocarbons (PAHs) as well as aromatic ethers, alkyl aromatic ketones (acetophenone, propiophenone, butyrophenone, and valerophenone), benzonitrile, aromatic esters, substituted nitrobenzenes, and alkyl anilines. For this subgroup A:

$$\log k'(10\text{mM } C_{14}\text{TAB}) = (0.67 \pm 0.01) \log P_{ow}$$

$$- 1.62 \quad (7)$$

$$n = 38, r = 0.9769, \text{S.E.} = 0.120$$

The second subgroup, B (middle line with open squares), contains benzyl alcohols which are HBD compounds with intermediate strength ($0.33 \leq \alpha \leq 0.40$). For this subgroup B:

$$\log k'(10\text{mM } C_{14}\text{TAB}) = (0.75 \pm 0.03) \log P_{ow} - 1.57 \quad (8)$$

$n = 7, r = 0.9953, \text{S.E.} = 0.026$

The third subgroup, C (upper line with filled squares), includes phenols which are the strongest HBD among all solutes ($\alpha \geq 0.54$) as well as aniline ($\alpha = 0.26$) and haloanilines ($\alpha = 0.31$) which have intermediate α values. For subgroup C:

$$\log k'(10\text{mM } C_{14}\text{TAB}) = (0.89 \pm 0.02) \log P_{ow} - 1.56 \quad (9)$$

$n = 15, r = 0.9882, \text{S.E.} = 0.074$

This is consistent with the LSER analysis [22], since the C_{14} TAB micelles are the most basic among four micellar systems and should have the strongest type B hydrogen bonding interaction with acidic phenols.

There is no congeneric behavior in the $\log k'$ vs. $\log P_{ow}$ plot for the SC-MEKC system. Only one line is adequate to describe the relationship between $\log k'$ vs. $\log P_{ow}$ (Fig. 2). This may be due to the similar hydrogen bonding patterns in 1-octanol and SC micelles [20,21].

Another reason for the differences in $\log k'$ vs. $\log P_{ow}$ relationships for SDS and C_{14} TAB as compared to SC systems can be due to dissimilar micelle structures. In general, micelles are dynamic aggregates with heterogeneous microenvironments. Solute experience different microenvironment polarities as they are localized in/on micelles. Solute-micelle interactions occur through different mechanisms such as surface adsorption, partitioning into the micellar core, and co-micellization. Thus, the operating interactive forces between various groups of solutes and micelles are different, and depend on the location of solutes in/on micelles. Roughly spherical SDS or C_{14} TAB micelles have a large aggregation number (e.g., around 60 for SDS in water at 25°C), while bile salts micelles (such as

SC) are much smaller with an aggregation number of 2–10. Intuitively, one can then conclude that there is a greater degree of heterogeneity in the SDS or C_{14} TAB micelle structure than in SC micelle structure. This leads to a larger degree of variation in retention behavior in the SDS or C_{14} TAB systems as compared to the SC-MEKC systems.

3.2. Effect of mixed micelles and modifiers

An inherent limitation of MEKC is the existence of an elution window [7–9]. All solutes would have to be separated between the elution of an unretained solute (with a migration time of t_{eo}) and the elution of micelles (with a migration time of t_{mc}). That is, separations of highly hydrophilic (i.e., very small P_{mw}) and highly hydrophobic compounds (i.e., very large P_{mw}) are not feasible in MEKC. This would also limit the range of hydrophobicity (or range of $\log P_{ow}$) that can be determined by MEKC. The application of MEKC for highly hydrophobic compounds, or those with a strong interaction with micelles, can be extended through a change in the type of surfactant, addition of modifiers such as organic solvents or cyclodextrins to the micellar solution, and/or the use of mixed micellar systems.

One such example is with corticosteroids. Note that seven of the nine corticosteroids listed in Table 1 are only moderately hydrophobic (with $\log P_{ow}$ values less than 2.0); only two compounds are very lipophilic and have $\log P_{ow}$ values 2.90 and 3.87. However, these compounds can not be separated by an SDS system, as they bind strongly to the SDS micelles and elute near or at t_{mc} . Many of the other 60 aromatic compounds with larger $\log P_{ow}$ values, can be easily separated in SDS-MEKC systems. In other words, retention behavior of corticosteroids in SDS-MEKC can not be fully explained by their hydrophobicity (or $\log P_{ow}$ scale). Their stronger interactions with SDS micelles than those with SC micelles are probably due to their large bulky size, the hydrogen bonding interactions between HBA corticosteroids (with several carbonyl and hydroxyl groups) and HBD, SDS

micelles; as well as larger phase ratio of SDS micelles than that of SC micelles at the same surfactant concentration [20]. In order to separate these compounds, the use of three different modifiers (acetonitrile, β -cyclodextrin, and urea) in SDS solutions and other types of individual and mixed bile salts surfactants were examined. The mixed micellar systems provided the best separations for a complex mixture of 17 corticosteroids with high structural similarities [23,24].

The results of $\log k'$ vs. $\log P_{ow}$ regression for nine corticosteroids in different MEKC systems are listed in Table 3. High correlations were observed with the individual and mixed bile salt

surfactants. The addition of SDS to the mixed bile salts systems resulted in lower correlations. The SDS systems with modifiers (ACN, β -cyclodextrin, and urea) do not offer correlations as high as bile salt surfactant MEKC systems. Apparently, sodium deoxycholate (SDC) and sodium cholate (SC) or mixtures of the two provide higher correlations than those of sodium taurocholate (STC) and sodium glycodeoxycholate (SGDC) or mixtures of the two. This may be due to more structural similarity between 1-octanol and SDC with SC, as compared to STC and SGDC which have peptide chains in the ionic head groups [24].

Table 3
Quantitative relationships between the $\log k'$ values of steroids in MEKC and their $\log P_{ow}$ values

System	$\log k' = a \log P_{ow} + b$				
	<i>a</i>	<i>b</i>	<i>n</i>	<i>r</i>	S.E.
<i>Individual surfactant systems</i>					
100 mM SC	0.490 (0.03) ^a	-0.505	9	0.981	0.092
100 mM SDC	0.491 (0.02) ^a	-0.113	9	0.988	0.071
100 mM STC	0.362 (0.03) ^a	-0.347	9	0.957	0.103
100 mM SGDC	0.459 (0.04) ^a	-0.208	9	0.967	0.114
<i>Mixed surfactant systems</i>					
50 mM SGDC, 50 mM SDS	0.648 (0.09) ^a	-0.233	9	0.910	0.279
50 mM STC, 50 mM SGDC	0.418 (0.03) ^a	-0.258	9	0.972	0.096
50 mM SDC, 50 mM SC	0.494 (0.03) ^a	-0.321	9	0.982	0.089
<i>SDS/modifier systems</i>					
100 mM SDS, 4 M Urea	0.802 (0.09) ^a	-0.246	8 ^b	0.950	0.156
100 mM SDS, 20 mM β -CD	0.655 (0.13) ^a	0.138	9	0.839	0.401
100 mM SDS, 5% ACN	0.884 (0.15) ^a	-0.232	8 ^b	0.902	0.252

^a 95% confidence interval.

^b *n* = 8 because progesterone coelutes with *t*_{mc} marker.

Abbreviations: see Table 2 and sodium deoxycholate (SDC), sodium taurocholate (STC), sodium glycodeoxycholate (SGDC), β -cyclodextrin (β -CD), acetonitrile (ACN). See Table 2 for definition of *n*, *r*, S.E.

3.3. Estimation of $\log P_{ow}$ from MEKC retention

The $\log P_{ow}$ values for 60 aromatic compounds were estimated from their retention in the SC-MEKC system (Fig. 5). The leave-one-out approach was used as the $\log P_{ow}$ for each compound in the set was predicted based on the $\log k'$ vs. $\log P_{ow}$ model (i.e., Eq. 2b), using the remaining 59 compounds. This process was repeated for all sixty compounds in the set. Note that some of the experimental errors for the estimation of $\log P_{ow}$ with MEKC techniques are associated with calculating capacity factor (k') when the migration time of a solute approaches t_{eo} or t_{mc} . Capacity factor in MEKC is calculated according to Eq. 10 [8,9].

$$k' = \frac{t_r - t_{eo}}{t_{eo} \left(1 - \frac{t_r}{t_{eo}}\right)} \quad (10)$$

where k' is the capacity of a solute, t_r is the migration time of the solute, t_{eo} is the migration time of an unretained solute, and t_{mc} is the migration time of micelles.

Another aspect of the QSRR models is the possibility of prediction of retention behavior from the chemical structure or physico-chemical properties. This can greatly reduce the amount

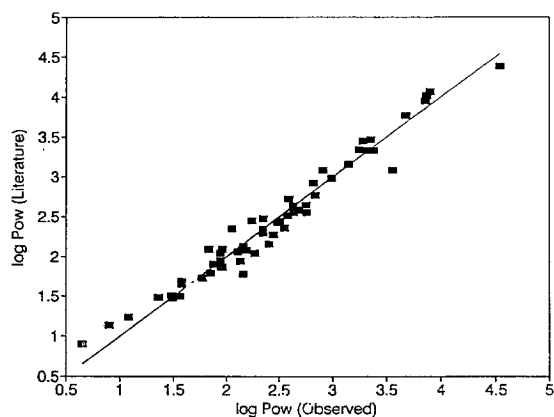


Fig. 5. Predicted hydrophobicity vs. literature values for hydrophobicity with SC (0.080 mM)-MEKC system for a group of 60 aromatic compounds using the leave-one-out technique.

of experimental effort in optimization of separations. Capacity factors of solutes in MEKC with SC and SDS micelles were predicted from their $\log P_{ow}$ values using Eq. 2a. An example of this application is the prediction of capacity factors of the test solutes in the SC-MEKC system from their hydrophobicity, as shown in Fig. 6. Not surprisingly, high correlation exists between the predicted and the observed capacity factors. The leave-one-out technique was also applied in this case. The prediction is not as accurate for the SDS system if the $\log k'$ vs. $\log P_{ow}$ model for the main set ($n = 60$) is used (see Table 2). This is due to the congeneric behavior in the SDS system. The situation can be improved by using the appropriate model for the congeneric solutes. Obviously, retention in LiP-FOS-MEKC system can not be accurately predicted from $\log P_{ow}$ due to the poor correlation.

3.4. Quantitative retention-activity relationships in MEKC

The relationships between retention in MEKC and biological activities for corticosteroids were also examined. Two types of biological responses that have been related to the solutes' hydrophobicity were used: small intestinal absorption in the rat [25] and binding to human serum albumin protein [26].

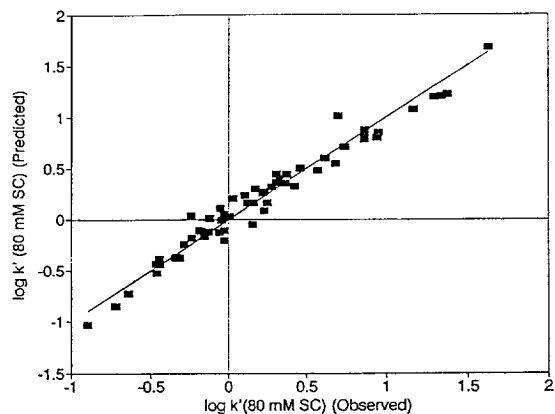


Fig. 6. Predicted capacity factors vs. observed capacity factors for MEKC system with 0.080 mM SC using the leave-one-out technique.

The absorption activity was in terms of the logarithm of % absorbed (A) divided by % non-absorbed (NA) [$\log (A/NA)$], in order to better reflect the solute partitioning process. As shown in Table 4, high correlations were observed between $\log (A/NA)$ and $\log k'$ using bile salt surfactants and mixed bile salt surfactants. The addition of SDS micelles to bile salt surfactants and addition of buffer additives (e.g., β -cyclodextrin) to SDS resulted in lower correlations between $\log (A/NA)$ and $\log k'$. Similar observations were found between $\log P_{ow}$ and $\log k'$ for these steroids with the same buffer conditions as shown in Table 3.

The relationships between binding of steroids to serum albumin protein and the logarithm of capacity factors in MEKC were also examined. The binding activity was in terms of the logarithm of % bound (B) divided by % free (F) [$\log (B/F)$]. As shown in Table 4, high correla-

tions were observed in most cases. The addition of β -cyclodextrin to SDS-MEKC system gave the worst correlations in quantitative retention-activity relationships (QRAR). Fig. 7 shows the predicted vs. observed values of $\log P_{ow}$, $\log (A/NA)$, and $\log (B/F)$ for corticosteroids that have all been predicted, using the leave-one-out approach, from the correlations with MEKC retention listed in Tables 3 and 4.

4. Conclusions

MEKC offers a number of advantages in QSAR studies for estimation of both biological activity and hydrophobicity based on solute retention. Our results clearly show that type of surfactant has a tremendous effect on the correlation between $\log P_{ow}$ and $\log k'$. However, the

Table 4
Quantitative relationships between $\log (A/NA)$ and $\log (B/F)$ of steroids and their $\log k'$ values in MEKC

System	$\log (A/NA) = a \log k'$				
	a	b	n	r	S.E.
100 mM SDC	1.592 (0.15) ^a	-1.453	8 ^b	0.957	0.234
50 mM SGDC, 50 mM STC	1.789 (0.17) ^a	-1.133	8 ^b	0.957	0.234
50 mM SGDC, 50 mM SDS	1.023 (0.15) ^a	-1.192	8 ^b	0.906	0.342
100 mM SDS, 20 mM β -CD	0.874 (0.20) ^a	-1.368	8 ^b	0.843	0.435
	$\log (B/F) = a \log k' + b$				
100 mM SGDC	0.992 (0.08) ^a	-0.244	9	0.966	0.119
50 mM SGDC, 50 mM STC	1.084 (0.10) ^a	-0.167	9	0.957	0.133
50 mM SGDC, 50 mM SDS	0.645 (0.07) ^a	-0.226	9	0.942	0.155
100 mM SDS, 20 mM β -CD	0.558 (0.09) ^a	-0.352	9	0.894	0.206

^a 95% confidence interval.

^b Absorption data not available for 6 α -methyl-prednisolone.

See Tables 2 and 3 for definitions of abbreviations. See Table 2 for definition of n , r . S.E. is the standard error of $\log (A/NA)$ or $\log (B/F)$ estimated.

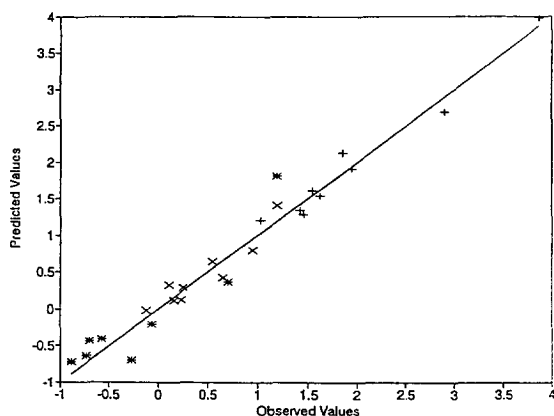


Fig. 7. Predicted values vs. literature values of hydrophobicity and biological activity for corticosteroids using the leave one out technique. (+) $\log P_{ow}$ values using a 0.100 M sodium deoxycholate system; (x) $\log (A/NA)$ values using a 0.050 M sodium taurocholate/0.050 M sodium glycodeoxycholate mixed system; (*) $\log (B/F)$ values using a 0.100 M sodium deoxycholate system.

concentration of the surfactant plays a minor role on the correlations. Bile salt surfactants have clear advantage over SDS or C_{14} TAB for estimation of $\log P_{ow}$ and in QSAR studies due to the very similar hydrogen bonding interaction patterns between bile salt MEKC systems and the 1-octanol–water system. On the other hand, in SDS and C_{14} TAB micellar systems, the congeneric problem is observed as different relationships between $\log k'$ and $\log P_{ow}$ are observed for various groups of solutes (with specific hydrogen bonding properties). The LSER analysis of retention behavior and 1-octanol–water partitioning is of great use in identifying the most suitable MEKC system for QSAR analysis. In this study, the test solutes were uncharged. Many of biologically active compounds of interest in QSAR studies are ionic at physiological pH. The migration behavior of ionizable compounds in MEKC is more complicated than that of uncharged solutes. However, the MEKC-based method can be easily extended to ionizable compounds using the retention models developed previously in this laboratory [27,28]. These studies are presently underway.

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