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Solute retention in micellar liquid chromatography

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ABSTRACT

A study of the retention behavior of aromatic compounds in micellar and hydro-organic mobile phases was undertaken to better understand the differences in retention and selectivity between micellar liquid chromatography (MLC) and reversed-phase liquid chromatography. The capacity factor for 21 aromatic compounds was measured on Microsorb C_{18} using both micellar and hydro-organic mobile phases. Through correlation analysis it was shown that solute retention in MLC is influenced in some measure by the net surface charge of the stationary phase as well as by the unusual nature of the micelle–solute interaction.

INTRODUCTION

In 1980, Armstrong and Henry [1] demonstrated that aqueous micellar solutions can be used as mobile phases in reversed-phase liquid chromatography (RPLC). They called this technique pseudo-phase or micellar liquid chromatography (MLC). Since the first report by Armstrong and Henry, a number of articles have appeared in the chemical literature focusing on the unique capabilities of MLC, *e.g.*, rapid gradient capability, enhanced luminescence detection, simultaneous separation of charged and neutral compounds, and low toxicity and cost, to name a few [2–8]. More than a hundred papers to date have been published on this subject.

Retention in MLC has been shown to be correlated to surfactant type and to the concentration of the surfactant (above the critical micelle concentration) in the mobile phase [9,10]. Solute retention in MLC generally decreases with increasing surfactant (*i.e.*, micelle) concentration, but the rate of decrease can vary considerably from one organic solute to the next. Equations relating the capacity factor (k') to the concentration of the micelles in the mobile phase have been developed by Armstrong and Nome [11] and Cline-Love and Arunyanart [12] based on a three-way partition model. The equations by Armstrong and Nome [11] and Cline-Love and Arunyanart [12] have been verified experimentally [13–15] for a large number of organic solutes.

As in RPLC, selectivity in MLC is controlled primarily by manipulation of the mobile phase composition. However, mobile phase–solute interactions in MLC are very different in nature from those in RPLC. To better understand the differences in retention and selectivity between the two techniques, a study of the retention behavior

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of aromatic compounds in micellar and hydro-organic mobile phases was undertaken. This paper, as a preliminary report of an on-going investigation, stresses the importance of solute–stationary phase interactions in MLC. Using a set of 21 aromatic compounds as retention probes (see Table I), we will show that differences in retention and selectivity do, in fact, exist between the two techniques. However, these differences can be attributed in some measure to changes in the net surface charge of the stationary phase caused by adsorbed surfactant.

EXPERIMENTAL

All high-performance liquid chromatographic (HPLC) measurements were made with a Rainin 81-20 M analytical HPLC system which incorporates two Rainin Rabbit HP pumps (Rainin Instruments, Woburn, MA, U.S.A.), an Apple Macintosh computer as the controller, a Model 7125 Rheodyne injection valve, and a Rainin Dynamax mixer. The detector was a Knauer variable-wavelength UV–visible spectrometer (Berlin, Germany). The analytical columns used were Rainin Microsorb 3-μm octyldecyldimethylsilane ODS (50 mm $\times$ 4.6 mm I.D.). A silica precolumn placed between the injector and the pumps was used to saturate the mobile phase with silicates. The analytical column and the mobile phase reservoir were water-jacketed and temperature-controlled.

The 21 mono-, di- and trisubstituted benzenes were obtained from Aldrich and were used as received. Sodium dodecylsulphate (SDS) and cetyltrimethylammonium bromide (CTAB) were obtained from BDH (99% purity). SDS was recrystallized in ethanol and dried in an oven at 65°C prior to chromatographic use, whereas, CTAB was used as received. HPLC-grade distilled water, HPLC-grade methanol and 1-propanol were obtained from J. T. Baker.

The micellar solutions were prepared in HPLC-grade distilled water. The methanol–water mobile phase was also prepared with HPLC-grade solvents. Both the micellar solutions and the methanol–water mobile phase were filtered twice through a 0.45-μm Nylon membrane filter to remove particulate matter. The solutions were also degassed prior to use. pH measurements on these solutions were made using a ChemTrix pH meter. The pH of each solution was approximately 6.30.

TABLE I

THE AROMATIC DATA SET

(1) Benzyl alcohol	(12) Chlorobenzene
(2) Benzaldehyde	(13) Bromobenzene
(3) 2,4-Dinitrophenol	(14) Ethylbenzene
(4) Benzonitrile	(15) Resorcinol
(5) Acetophenone	(16) Catechol
(6) Nitrobenzene	(17) Phenol
(7) *p*-Nitroanisole	(18) *p*-Nitrophenol
(8) Methylbenzoate	(19) *o*-Chlorophenol
(9) Anisole	(20) *o*-Bromophenol
(10) Benzene	(21) 2,4-Dichlorophenol
(11) Toluene	

The void volume of the system was determined by injecting different solutions such as methanol, methanol–water, or water onto the Microsorb columns. Void volume measurements obtained for micellar mobile phases were comparable to the values obtained for methanol–water mobile phases. This volume, approximately 0.55 ml, was used for all k' calculations. The k' values determined in this study were averages of at least triplicate determinations. All capacity factor measurements were made at a flow-rate of 1.0 ml/min. The k' values were measured at 25°C for SDS and 35°C for CTAB. (Since the Kraft point of CTAB is 23°C, it was necessary to carry out the CTAB studies at a higher temperature.)

During the course of this study, solutions of surfactant containing small amounts of organic modifier, *e.g.*, 2% 1-propanol or 20% 1-propanol (v/v), were used as mobile phases. The presence or absence of surfactant aggregation (*i.e.*, micelles) in these so-called hybrid mobile phases was determined by conductometric titration [16]. Distilled water or distilled water with 2% 1-propanol or 20% 1-propanol was added to a thermostated and stirred cell. A surfactant solution prepared in the same medium was titrated against the solution in the cell. Conductance measurements were then taken periodically after addition of the titrant using a dipping electrode (nominal cell constant of 1 cm^{-1}) and a Cole-Palmer conductivity meter. If the titration curve had a sharp endpoint, the presence of micelles was so indicated in the medium. The endpoint presumably denotes the critical micelle concentration (CMC) of the surfactant in the medium (see Table II).

RESULTS AND DISCUSSION

The first step in the study was to characterize the retention behavior of the 21 aromatic compounds on Microsorb C_{18} using a hydro-organic mobile phase [methanol–water (50:50, v/v)] and a micellar mobile phase [0.05 *M* SDS with 2% 1-propanol (v/v)]. Propanol was added to the SDS solution to improve the chromatographic efficiency of the C_{18} column. It is known that poor column efficiency in MLC is caused by slow mass transfer due to poor wetting of the stationary phase [17]. This is an especially troublesome problem for C_{18} columns. The presence of an organic solvent such as 1-propanol in the mobile phase is known to provide the wetting that is needed for good mass transfer. For SDS mobile phases, small amounts of 1-propanol or an equivalent organic solvent are crucial to ensure reproducible

TABLE II

SUMMARY OF THE RESULTS FROM THE CONDUCTOMETRIC TITRATION EXPERIMENTS PERFORMED USING MICELLAR AND TERNARY (*I.E.*, HYBRID) MOBILE PHASES

System	Temperature (°C)	Detectable endpoint	CMC (*M*)
0.05 *M* SDS	25	Yes	0.0082
0.05 *M* SDS with 2% *n*-propanol	25	Yes	0.0040
0.05 *M* CTAB	35	Yes	0.0019
0.05 *M* CTAB with 2% *n*-propanol	35	Yes	0.0016
0.05 *M* CTAB with 20% *n*-propanol	35	No	–

chromatography. (In fact, workers in our laboratory were unable to generate reliable retention data on short Microsorb C_{18} columns using SDS micellar solutions that did not contain small amounts of 1-propanol.) With regard to this so-called hybrid mobile phase, it was evident on the basis of conductivity measurements (see Table II) that micelles were present in the surfactant solution. In fact, McGreevy and Schecter [18] have found that micelle size is not affected by the addition of long-chain alcohols, *e.g.*, 1-butanol, over the concentration range 0–0.162 *M*.

The retention data generated with the methanol–water mobile phase and the SDS micellar mobile phase were analyzed using a form of the Collander equation. In Fig. 1, log *P* (the log of the octanol–water partition coefficient) is plotted against log *k'* for the methanol–water mixture. The log *P* values were obtained from the Pomona College Medicinal Chemistry Data Bank [19]. An examination of Fig. 1 reveals a very interesting result: the compounds in the data set can be divided into two groups. The first group of compounds (*i.e.*, group B) consists entirely of phenols (numbers 15–21), whereas the second group (*i.e.*, group A) is mainly mono-substituted benzenes (numbers 1, 2 and 4–14). When the correlation coefficient was computed for each group, the r^2 value was 0.97 for group A and 0.98 for group B. The r^2 value for the whole data set (excluding 2,4-dinitrophenol) was 0.86. Compound 3 (2,4-dinitrophenol) has a pK_a of 4.07. Because the pH of the mobile phase is 6.30, we would expect 2,4-dinitrophenol to exist principally in the anionic form. Therefore, 2,4-dinitrophenol would be too polar to partition into the stationary phase and would be expected to elute off the column with the dead marker.

The degree of correlation between log *P* and log *k'* was also determined for the compounds using the SDS mobile phase. In Fig. 2, a plot of log *P versus* log *k'* is shown

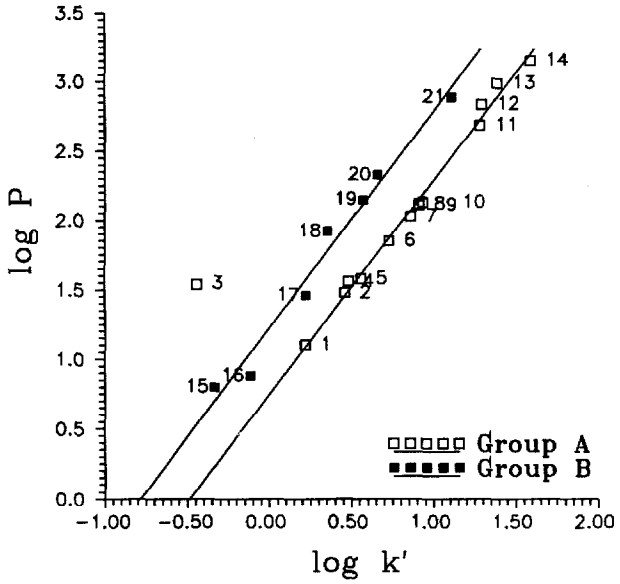

Fig. 1. Plot of log *P versus* log *k'* for the 21 aromatics, *e.g.*, 1 is benzyl alcohol, 2 is benzaldehyde (see Table I). The mobile phase consisted of methanol–water (50:50).

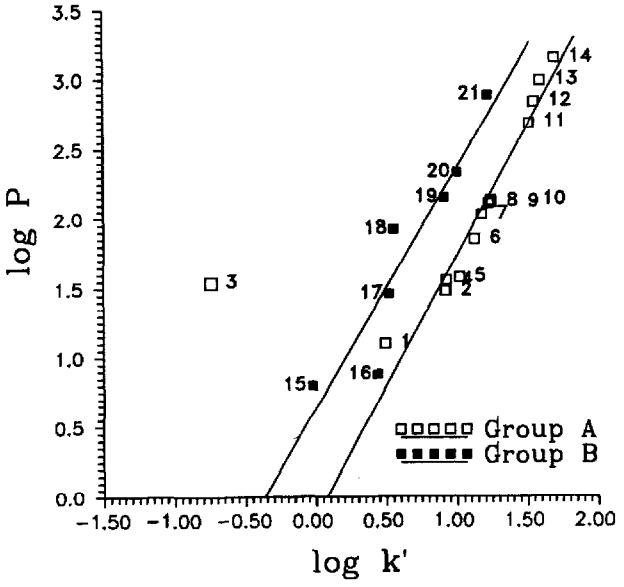

Fig. 2. Plot of log P *versus* log k' for the 21 aromatics. The mobile phase consisted of 0.05 M SDS and 2% 1-propanol (v/v).

for the 21 aromatic compounds. Again, the aromatics can be divided into two groups; 2,4-dinitrophenol again elutes off the column with the dead marker. In fact, the only difference between the two data sets is that catechol lies in group A instead of group B. (There is no difference in the elution order of the compounds and the same type of dichotomy exists in the data.) Table III provides a statistical summary of these results. The slopes of the lines drawn through the sets of points are approximately equal for each data set.

The similarity between the two data sets is surprising in view of the reported differences in selectivity between micellar mobile phases and hydro-organic mobile phases [20,21]. To better understand the reasons for the similar results, we measured k' on a C_{18} Microsorb column for the aromatic compounds using five different SDS

TABLE III

STATISTICAL SUMMARY OF THE RESULTS OBTAINED FROM THE METHANOL–WATER AND SDS DATA SETS

Mobile phase	Group	Slope	Intercept	r^2
Methanol–water	A	1.54	0.75	0.97[a]
	B	1.56	1.22	0.98
SDS	A	1.87	−0.14	0.98[a]
	B	1.73	0.63	0.84

[a] 2,4-Dinitrophenol was excluded from the calculation.

mobile phases: (1) 0.01 *M* SDS with 2% 1-propanol; (2) 0.025 *M* SDS with 2% 1-propanol; (3) 0.05 *M* SDS with 2% 1-propanol; (4) 0.10 *M* SDS with 2% 1-propanol; and (5) 0.15 *M* SDS with 2% 1-propanol. Next, we correlated k' to surfactant concentration using the equation developed by Cline-Love and Arunyanart [12]:

$$\frac{1}{k'} = \frac{[\mathrm{M}]K_2}{\varphi[\mathrm{L_s}]K_1} + \frac{1}{\varphi[\mathrm{L_s}]K_1} \tag{1}$$

where [M] is the concentration of surfactant, K_2 is the solute–micelle binding constant, φ is the chromatographic phase ratio, $[\mathrm{L_s}]$ is the concentration of ligate on the stationary phase, and K_1 is the solute–stationary phase binding constant. A plot of $1/k'$ *versus* [M] should result in a straight line. In fact, excellent linearity was observed for 20 of the 21 aromatic compounds ($r^2 > 0.985$). (The exception, of course, was 2,4-dinitrophenol which came out with the dead marker.) Table IV lists the values for K_2 and the intercepts, *i.e.*, $\varphi[\mathrm{L_s}]K_1$, for 20 of the 21 aromatic compounds. (K_2 was obtained by dividing the slope by the intercept and multiplying by the aggregation number.) The values obtained for K_2 were, by and large, in good agreement with previously published literature values [22].

Treiner [23] has previously reported that an excellent linear correlation exists between log *P* and log K_2 for aliphatic compounds. Therefore, we attempted to correlate log *P* to log K_2 for the aromatics. In Fig. 3, a plot of log *P* *versus* log K_2 is shown for the aromatic compounds. A nice straight line can be drawn through the data

TABLE IV

SODIUM DODECYLSULPHATE WITH 2% 1-PROPANOL

Compound	Slope	Intercept	K_2[a]	$\varphi[\mathrm{L_s}]K_1$
Benzyl alcohol	1.5	0.19	520	5.2
Benzaldehyde	0.77	0.65	780	15
Benzonitrile	0.86	0.07	810	14
Acetophenone	0.69	0.043	1100	23
Nitrobenzene	0.70	0.41	1120	25
p-Nitroanisole	0.77	0.026	2000	39
Methyl benzoate	0.62	0.020	2040	50
Anisole	0.67	0.028	1600	36
Benzene	0.72	0.020	2400	51
Toluene	0.51	0.0078	4300	130
Chlorobenzene	0.49	0.0068	4700	150
Bromobenzene	0.47	0.0045	7000	220
Ethylbenzene	0.42	0.0022	13 000	450
Resorcinol	4.3	1.1	260	0.89
Catechol	1.7	0.36	300	2.7
Phenol	1.5	0.20	500	4.9
p-Nitrophenol	1.9	0.13	980	7.7
o-Chlorophenol	1.2	0.055	1500	18.2
Bromophenol	1.2	0.037	2100	27
2,4-Dichlorophenol	1.0	0.007	9000	140

[a] K_2 is computed by multiplying 66 (the aggregation number for SDS) by the ratio of the slope to the intercept.

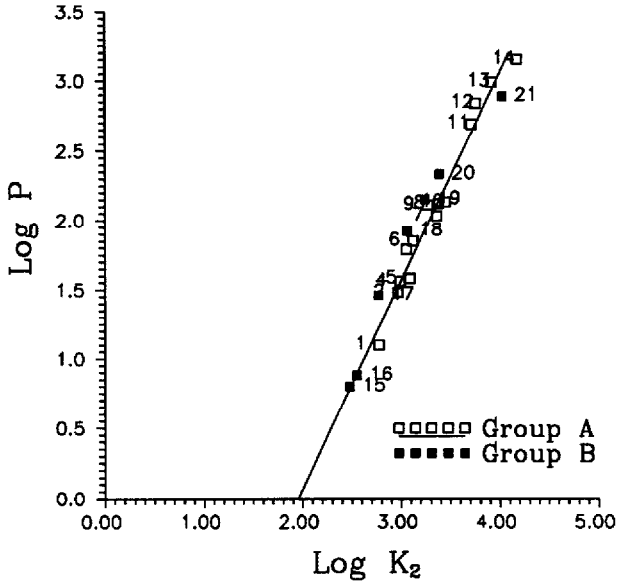

Fig. 3. Plot of log P *versus* log K_2 for the 21 aromatics. SDS was the surfactant.

points ($r^2 = 0.96$). Evidently, the dichotomy present in the log P *versus* log k' plot of SDS cannot be attributed to a mobile phase effect, *i.e.*, selectivity by the surfactant aggregate towards the phenols. Nor can the differences in the chromatographic behavior of the phenols from that of the other aromatic compounds tested be explained on the basis of ion-interaction or ion-pair chromatography [24,25]. With the exception of 2,4-dinitrophenol, the k' value of the other phenols did not change when the pH of the 0.05 M SDS mobile phase was adjusted to 4.30 or 3.00, via addition of small amounts of H_2SO_4.

In all likelihood, the silanol groups on the bonded phase surface are responsible for the differences in the chromatographic behavior between the phenols and the other aromatic compounds tested. Minick *et al.* [26] have observed this effect in RPLC with C_{18} and C_8 columns and have shown that it can be minimized by adding trace quantities of *n*-decylamine and 1-octanol to the hydro-organic eluent. Indeed, the sensitivity of the alkyl bonded phase to differences in hydrogen bonding properties of the solutes [27–29] is quite pronounced, more so than for the octanol–water system.

If the dichotomy of results observed for SDS is also due to unhindered silanol groups on C_{18}, then one should expect the same dichotomy from the plot of log P *versus* log K_1. In Fig. 4, a plot of log P *versus* log $\varphi[L_s]K_1$ is shown for the 21 aromatic compounds. The phase ratio and the concentration of the ligate on the stationary phase are constants that are characteristic of the chromatographic system. Therefore, this plot defines the relationship between the octanol–water partition coefficient and the solute–stationary phase binding constant. The similarity between Figs. 2 and 4 is truly remarkable. Therefore, we conclude that the stationary phase is responsible for the dichotomy present in the log P *versus* log k' plots shown.

Our studies of micellar mobile phases in RPLC, however, were not limited to

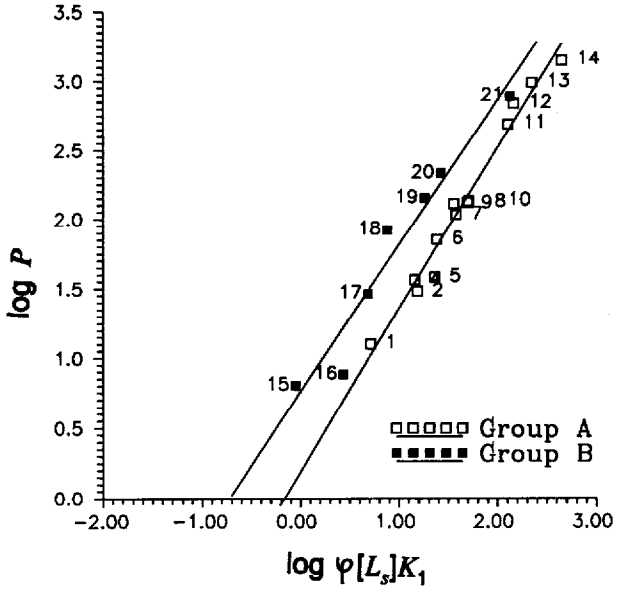

Fig 4. Plot of log P *versus* log $\varphi[L_s]K_1$ for the 21 aromatics. SDS was the surfactant.

only anionic surfactants. In Fig. 5, a plot of log P *versus* log k' is shown for the 21 aromatic compounds using a 0.05 M CTAB solution as the mobile phase. (1-Propanol was not added to the 0.05 M CTAB solution because poor column efficiency was not as serious a problem.) From Figs. 2 and 5, it is evident that the plot of log P *versus* log k'

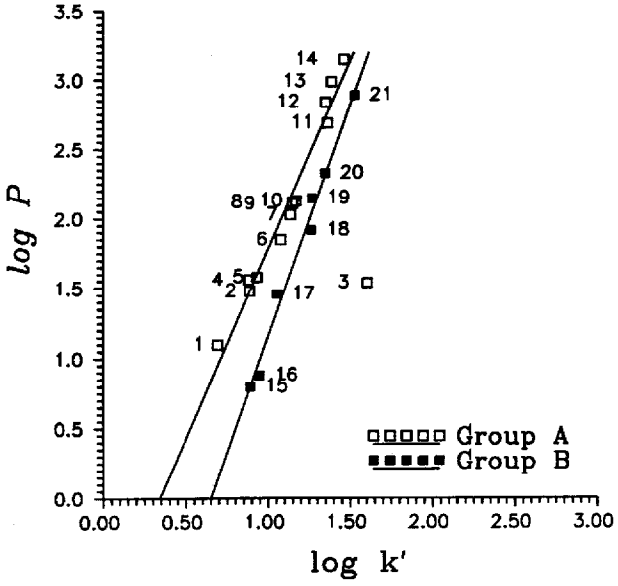

Fig. 5. Plot of log P *versus* log k' for the 21 aromatics. The mobile phase consisted of 0.05 M CTAB.

for SDS (see Fig. 2) is different from the plot of log P *versus* log k' for CTAB. In the case of CTAB, group A is on the left and group B is on the right. For SDS, group A is on the right and group B is on the left. In addition, for CTAB, 2,4-dinitrophenol is strongly retained by the column, probably due to ion pairing with the adsorbed surfactant.

To better understand the reasons for the differences between SDS and CTAB, we again found it necessary to measure k' on C_{18} Microsorb for the aromatic compounds using a set of six different mobile phases: (1) 0.005 M CTAB; (2) 0.01 M CTAB; (3) 0.25 M CTAB; (4) 0.05 M CTAB; (5) 0.10 M CTAB; and (6) 0.20 M CTAB. Again, k' was correlated to surfactant concentration using the equation developed by Cline-Love and Arunyanart [12]. The agreement between $1/k'$ and [M] was very good. (In fact, $r^2 > 0.985$ for all 21 aromatic compounds.) Table V lists the values for K_2 and $\varphi[L_s]K_1$. Again, the values that were obtained for K_2 were, by and large, in good agreement with previously published literature values [22].

We again correlated log P to log K_2 for the aromatics. In Fig. 6, a plot of log P *versus* log K_2 is shown. The similarity between Figs. 6 and 5 is striking. Evidently, the differences in chromatographic behavior between the phenols and the other aromatic compounds tested can be attributed to a mobile phase effect, *i.e.*, selectivity by the surfactant aggregate towards the phenols. This selectivity is probably the result of a secondary equilibrium process involving the transfer of a proton from the phenol to the water molecules in the Stern region of the micelle. It is well known that the charge

TABLE V

CETYLTRIMETHYLAMMONIUM BROMIDE

Compound	Slope	Intercept	K_2[a]	$\varphi[L_s]K_1$
Benzyl alcohol	1.2	0.089	1000	11
Benzaldehyde	0.86	0.054	1200	18
2,4-Dinitrophenol	0.73	0.0021	28 000	490
Benzonitrile	0.89	0.050	1400	20
Acetophenone	0.82	0.045	1400	22
Nitrobenzene	0.82	0.025	2600	41
p-Nitroanisole	0.84	0.014	4500	70
Methyl benzoate	0.77	0.019	3200	52
Anisole	0.77	0.020	2900	49
Benzene	0.75	0.021	2800	48
Toluene	0.66	0.0078	6500	130
Chlorobenzene	0.67	0.0065	8100	150
Bromobenzene	0.67	0.0039	13 000	250
Ethylbenzene	0.58	0.0038	12 000	260
Resorcinol	1.3	0.036	2700	28
Catechol	1.05	0.028	2900	35
Phenol	0.93	0.024	3000	41
p-Nitrophenol	0.72	0.0043	13 000	230
o-Chlorophenol	0.69	0.0058	9200	170
Bromophenol	0.69	0.0029	19 000	340
2,4-Dichlorophenol	0.52	0.00037	110 000	2700

[a] K_2 is computed by mutiplying 78 (the aggregation number for CTAB) by the ratio of the slope to the intercept.

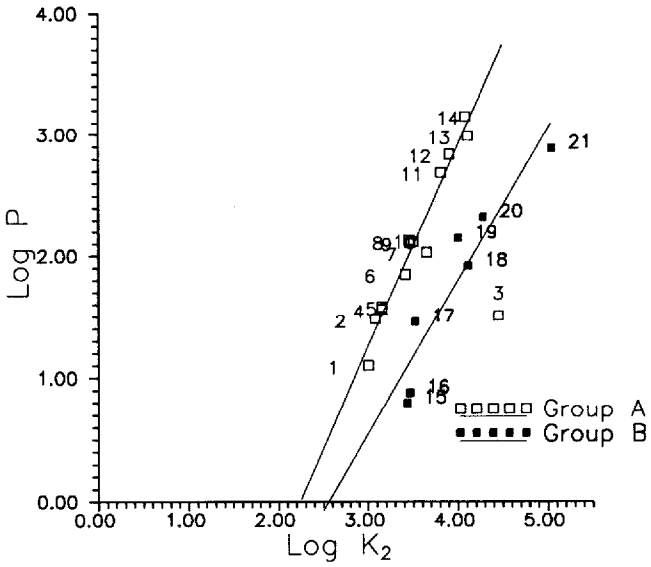

Fig. 6. Plot log P *versus* log K_2 for the 21 aromatics. CTAB was the surfactant.

on a cationic surfactant micelle will influence the pK_a value of an incorporated guest molecule [30]. A decrease of 0.5 to 3.0 in the pK_a value of dissociable amphiphiles will occur. (For anionic surfactants, the situation is reversed. In other words, an increase of 0.5 to 3.0 in the pK_a value will occur.) These shifts can be rationalized using simple electrostatic theory, *e.g.*, surface potential, low dielectric constant at the micellar surface [31].

However, the differences in selectivity between CTAB and SDS for the compounds tested cannot be attributed only to a shift in the pK_a values of the phenols. Fig. 7 shows a plot of log P *versus* log $\varphi[L_s]K_1$ for the CTAB data: the similarity between Figs. 7 and 5 is evident. When Fig. 7 is compared to Fig. 4, one has to conclude that the interaction of the solute with a CTAB-coated C_{18} phase is different from that of a SDS-coated C_{18} phase. This difference is probably due to a change in the surface charge of the stationary phase. In the case of CTAB, the chromatographic surface possesses a net positive charge due to the adsorbed CTAB monomer, whereas, in the case of SDS, the overall charge on the C_{18} surface is negative due to adsorbed SDS.

Although the role of the surfactant in the mobile phase has been extensively studied, the modification of the stationary phase by adsorbed surfactant has been investigated to a lesser extent [32–34]. Borgerding and Hinze [35] have observed that surfactant modification can cause changes in the selectivity and polarity of the stationary phase. Perhaps surface modification is also responsible for the similarity between aqueous micellar solutions and surfactant–water–propanol ternary mixtures with respect to the retention behavior of an homologous series. For example, Khaledi [20] noted that the addition of up to 20% of 2-propanol (v/v) to an SDS micellar mobile phase has a negligible effect on the hydrophobic selectivity of this mobile phase. However, we know that micelles will not be present in a solution of 0.05 M SDS with 20% 1-propanol (see Table II). Perhaps the similarity between aqueous micellar

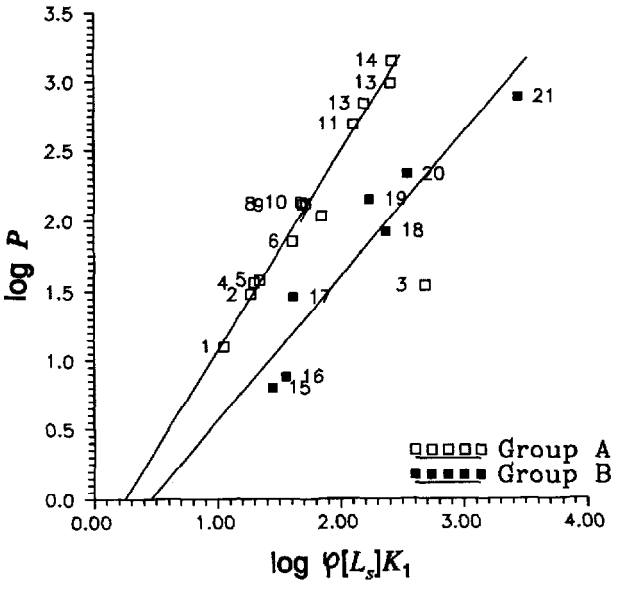

Fig. 7. Plot of log P *versus* log $\varphi[L_s]K_1$ for the 21 aromatics. CTAB was the surfactant.

solutions and the ternary mixtures is due in some measure to a modification of the surface of the stationary phase by the adsorbed surfactant.

CONCLUSION

Solute–stationary phase interactions in MLC are very important. It is possible that some of the reported differences in selectivity between MLC and RPLC with hydro-organic mobile phases are due in some measure to the modification of the surface of the stationary phase by adsorbed surfactant. The surface charge, as well as the structure of the C_{18} stationary phase, is altered by the adsorbed surfactant monomer. The results of this study also demonstrate that differences do exist in selectivity between micellar mobile phases and traditional hydro-organic mobile phases due to the unusual nature of the micelle–solute interaction.

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