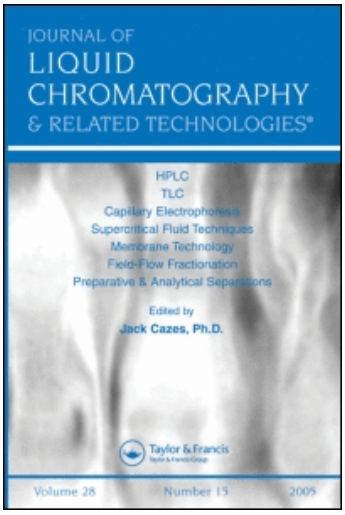

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

New Perspectives in Micellar Liquid Chromatography

Michael F. Borgerding[a]; Ronald L. Williams Jr.[a]; Willie L. Hinze[a]; Frank H. Quina[b]
[a] Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina [b] Instituto de Quimica Universidade de Sao Paulo, Sao Paulo, Brasil

To cite this Article Borgerding, Michael F. , Williams Jr., Ronald L. , Hinze, Willie L. and Quina, Frank H.(1989) 'New Perspectives in Micellar Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 12: 8, 1367 — 1406
To link to this Article: DOI: 10.1080/01483918908049512
URL: http://dx.doi.org/10.1080/01483918908049512

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(8), 1367-1406 (1989)

NEW PERSPECTIVES IN MICELLAR LIQUID CHROMATOGRAPHY

MICHAEL F. BORGERDING[1],
RONALD L. WILLIAMS, JR. [1], WILLIE L. HINZE[1]*,
AND FRANK H. QUINA[2]
[1]*Department of Chemistry*
Wake Forest University
P.O. Box 7486
Winston-Salem, North Carolina 27109
[2]*Instituto de Quimica*
Universidade de Sao Paulo
Sao Paulo, Brasil

ABSTRACT

This paper will summarize several new findings obtained in our laboratory on the use of micellar mobile phases in liquid chromatography. The topics to be addressed include (i) stationary phase modification by the mobile phase surfactant in micellar liquid chromatography, (ii) investigation of the retention mechanism in micellar liquid chromatography (MLC) using an alkylbenzene homologous series, (iii) evaluation of the effects of organic additives upon retention and efficiency in MLC, and (iv) preliminary characterization of several new classes of surfactant molecules for use in MLC. The information gained from these studies provides new insights into the dynamics of MLC and demonstrates their potential usefulness in several new separation applications including the resolution of optical isomers.

INTRODUCTION

During the past 10 years, the utilization of aqueous micellar solutions (i.e. solutions containing a surfactant at a concen-

tration above its critical micelle concentration (CMC)) as the mobile phase in liquid chromatography (1,2) has generated considerable attention and been the focus of numerous studies. This general topic has been the subject of several fine reviews (3-10). The purpose of this article is to briefly summarize the results of our recent investigations which shed light on the nature of micellar liquid chromatography (MLC) separation process. Research discussed includes: the study of stationary phase modification by the mobile phase surfactant, elucidation of the MLC retention mechanism operative in nonionic MLC, characterization of the effects of different organic additives as MLC mobile phase modifiers, definition of the origins of poor chromatographic efficiency consistently observed in MLC, and application of unconventional micellar-forming molecules in MLC. It is hoped that this discussion will spark renewed interest in MLC and its unique capabilities.

EXPERIMENTAL

Apparatus

A Perkin-Elmer 240-C Elemental Analyzer equipped with an autosampler, AD-6 autobalance, and data station was used for the determination of % carbon. Surface area and pore volume measurements were made with a Micrometrics Digisorb 2600 (11,12).

The HPLC system employed for the homologous series work was constructed of Waters components and consisted of a M6000A pump, Waters Intelligent Sample Processor, Model 441 fixed UV detector, and Model 720 controller. Data acquisition and integration were performed with Computer Automated Laboratory Systems software and HP 1000 hardware. All data on this system were acquired at a flow rate of 1.0 mL/min and a detector wavelength of 254 nm using a Waters Resolve 5 micron C-18 spherical packing stainless steel 3.9 mm x 15 cm column (11,13).

All other chromatographic work reported was obtained using another Waters system consisting of an automated Model 680 con-

troller, Model 510 pumps, UK-6 injector, and Model 481 LC spectrophotometric detector. The columns utilized in this phase of the work were 100 x 4.6 mm stainless steel packed with 5 micron C-18 spherical particles and were obtained from Advanced Separation Technologies, Inc. (14,15).

Materials

The surfactants, Brij-35 (polyoxyethylene(23)dodecanol), Brij-22 (polyoxyethylene(10)dodecanol), NaLS (sodium dodecylsulfate), CTAC (hexadecyltrimethylammonium chloride), and NaDC (sodium deoxycholate) were used as received from Fisher Scientific Co., Sigma Chemical Co., Bio-Rad or Boehringer Mannheim Biochemicals, Eastman Kodak Co., and Kodak or Sigma, respectively. All test solutes employed were obtained from Kodak or Aldrich and had stated purities of 96% or greater and were used as received. The HPLC water utilized was either Fisher HPLC grade or in-house prepared distilled and de-ionized with a Barnstead NANOpure system. The column packing used in the stationary phase characterization work was Resolve C-18 which was purchased by special agreement from Waters Associates.

Methods

Surfactant Isotherms. Surfactant adsorption isotherms were constructed by measuring the carbon content of Resolve C-18 packing material after exposure to various amounts of the different surfactants examined and converting this data to the amount (mg) of surfactant sorbed per gram of Resolve C-18. To prepare the samples, the Resolve C-18 was equilibrated with aqueous surfactant test solutions at a ratio of 1 gram of C-18 packing per 25 mL of test solution. All samples were agitated for approximately 3 hours on a wrist action shaker with a total surfactant exposure period of 1 day. Next, each sample was vacuum filtered onto a nylon 66 membrane filter, transferred to a sample vial, and dried in a vacuum oven at 60° C for at least 48 hours. After drying, the carbon content of each sample was determined according to

standard procedures and the amount of surfactant sorbed per gram of Resolve C-18 calculated (11,12).

Nitrogen Porosimetric Determination of Stationary Phase Surface Areas and Pore Distributions. The surface area and pore volume distribution measurements were made using the Digisorb 2600, with sample preparation as described above. Standard adsorption and desorption procedures were performed under computer control to develop the nitrogen adsorption isotherm at 77 K. The surface areas, S_{BET}, were calculated using the BET equation from adsorption isotherm data between relative pressures of 0.05 and 0.21 (11,12). Pore volume distributions and the cumulative pore volume, CPV, were also calculated from the adsorption isotherm data for pore diameters between 20 and 600 Å, assuming cylindrically shaped pores, using the method of Barrett et al (17).

Other Procedures. The chromatographic phase ratio, φ, i.e. ratio of the stationary phase volume, V_s, to that of the mobile phase, V_m, was calculated from equation 1,

$$\varphi = \frac{W[CPV_s - CPV_b]}{V_m} \qquad \text{(eq. 1)}$$

where: W is the weight of the packing material in the column, CPV_s is the cumulative pore volume of unbonded Resolve silica, and CPV_b is the cumulative pore volume of the bonded C-18 Resolve packing material before or after exposure to the surfactant (13). The samples were prepared as described in the previous section. No significant differences were observed between cumulative pore volumes calculated from the adsorption isotherm and from the desorption isotherm (11,13).

The capacity fator, k', was calculated in the usual manner using either uracil, nitrite ion, or tetramethylammonium ion as void markers. The chromatographic efficiency was calculated using the manual procedure of Foley and Dorsey (18), equation 2,

$$N = \frac{41.7(t_r/W_{0.10})^2}{(B/A) + 1.25} \qquad \text{(eq. 2)}$$

where: t_r is the solute retention time, $W_{0.10}$ is the peak width measured at 10% peak height, and B/A is the peak asymmetry factor.

The different pseudophase partition coefficients in MLC, P_{mw}, P_{sw}, and P_{sm} which represent the solute partition coefficients between the micellar phase and water, stationary phase and water, and micellar phase and stationary phase, respectively, were determined using a modified form of the Armstrong-Nome treatment (4,19), equation 3:

$$\frac{1}{k'} = \frac{1}{\varphi}\left[\frac{\bar{v}[P_{mw} - 1]C_m}{P_{sw}} + \frac{1}{P_{sw}}\right] \qquad \text{(eq. 3)}$$

where φ is the chromatographic phase ratio, $\bar{v}$ is the molar volume of the surfactant, and C_m is the concentration of micellized surfactant. The intercept of a plot of $1/k'$ vs. C_m yields P_{sw} while the slope/intercept ratio equals P_{mw}. The ratio of P_{sw} to P_{mw} gives P_{sm} (19).

More extensive information on the experimental procedures and systems mentioned in this section is presented elsewhere (11-16).

RESULTS AND DISCUSSION

Micellar Systems Examined.

Table 1 lists the different micelle-forming surfactants examined in this work. Our studies have included examination of nonionic (Brij's), anionic (NaLS), and cationic (CTAC) surfactant micellar systems. These were selected due to their previous utilization in MLC (3-10). The bile salt sodium deoxycholate (NaDC), an anionic micelle-forming surfactant, has not been previously employed in micellar liquid chromatography. However, it is a chiral surfactant and can form therefore chiral micelles. Consequently, the possibility of separating optical isomers with this chiral micellar mobile phase exists.

In addition, the micellar parameters, i.e. CMC and aggregation number, N, for some of these micellar systems in water alone and in some aqueous-alcoholic solutions are presented in Table 1. It is important to note the fact that the presence of additives in aqueous surfactant solutions can alter both of these two mi-

TABLE 1

Micellar Characteristics of the Surfactants Utilized in this Investigation[a]

Name (Abbreviation)	Conditions	CMC, mM	N
Polyoxyethylene(23)dodecanol (Brij-35)	in H_2O	0.10	40
	+ 5% EtOH	1.25	32
	+ 10% EtOH	1.50	29
Polyoxyethylene(10)dodecanol (Brij-22)	in H_2O	0.09	97
Sodium Dodecylsulfate (NaLS)	in H_2O	8.1	62
	+0.10 M EtOH	7.7	64
	+0.10 M PrOH	7.4	--
	+0.10 M BuOH	5.4	--
	+0.10 M PeOH	3.0	54
Hexadecyltrimethylammonium Chloride (CTAC)	in H_2O	1.3	78
Sodium Deoxycholate (NaDC)	in H_2O	5.4[b]	--
	+0.01 M NaCl	5.0	4.3
	+0.15 M NaCl	2.4	8.0
	+0.59 M NaCl	1.5	11.7

[a]Data taken from References 10-16. [b]The CMC and N parameters for NaDC are dependent upon the concentration of the NaDC, pH, and ionic strength.

cellar parameters. Also, the dynamics and properties of such additive-modified micellar aggregates can be significantly altered compared to that of the regular normal aqueous micelles (10,14).

Stationary Phase Modification in Micellar Liquid Chromatography.

Although mobile phase phenomenon such as pseudophase partitioning and many different surfactant mobile phase compositions have been extensively studied (3-10), investigations of the potential for significant stationary phase modification by the mobile phase surfactant have been limited (10-12,16,20-23). The extent and nature of potential stationary phase modification in MLC are important for several reasons: (1) stationary phase modification may be constant above the CMC (6,7), (2) reduced chromatographic efficiency typically observed in MLC may be related to the extent of surfactant sorption (10-12,16,20,23), and (3) the adsorbed surfactant on the stationary phase may alter the chemical nature of the stationary phase, hence solute retention (14,21).

Figure 1 shows the adsorption isotherms for nonionic Brij-35 and anionic NaLS on C-18 Resolve packing. As can be seen, the amount of adsorbed surfactant initially increases very rapidly, followed by a more gradual increase at higher equilibrium surfactant concentrations. Although it is not easily discerned from the Figure, adsorption continues well beyond the CMC for both surfactants with 1 $\mu mol/m^2$ Brij-35 or NaLS sorbed on the C-18 bonded phase. Berthod and co-workers have also reported adsorption isotherms of ionic surfactants on various stationary phases and noted that in many instances additional surfactant adsorption occurred above the CMC (20-23). Thus, the assumption often quoted in the literature that adsorption is constant above the CMC because the amount of free surfactant monomer is constant (6,7) is clearly not correct for all surfactant micelle - stationary phase combinations (12,16,20). In fact, Sasaki et al have reported in the micellar literature that the free monomer

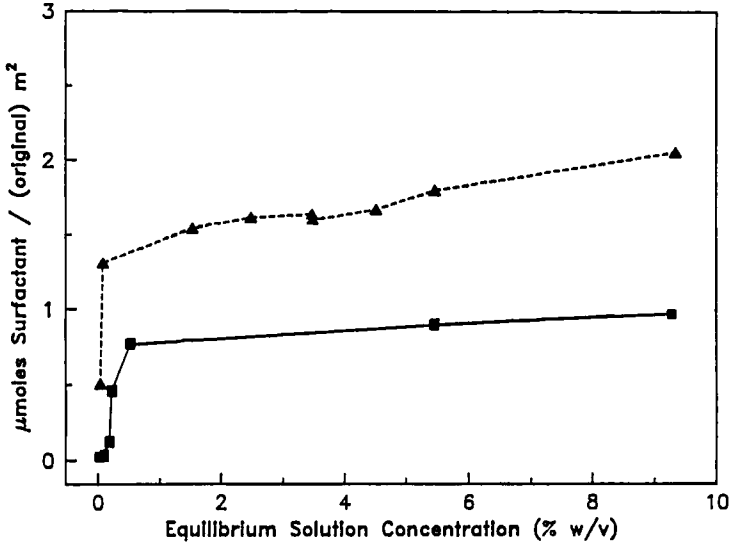

FIGURE 1. Adsorption isotherms for nonionic Brij-35 (▲) and anionic NaLS (■) surfactants on Resolve C-18 packing material.

concentration of nonionic surfactants continues to increase well above the CMC (24). Consequently, the reported ability to perform gradient elution in MLC (by increasing the surfactant micelle concentration) without re-equilibration of the chromatographic column (6,7) does not appear to be applicable to all charge-type surfactant micellar systems (11).

A decrease in the C-18 column packing material surface area was observed concomitant with the observed increase in adsorbed surfactant. The BET surface area was found to decrease about 60% for both the nonionic and anionic surfactant examined, which raises a question as to the altered nature of the pore shape in surfactant modified stationary phases. The pore shape of the surfactant modified stationary phase was investigated by comparison of hysteresis loops constructed from the sorption/desorption isotherms for untreated and surfactant modified Resolve C-18 material to hysteresis loops previously reported by de Boer

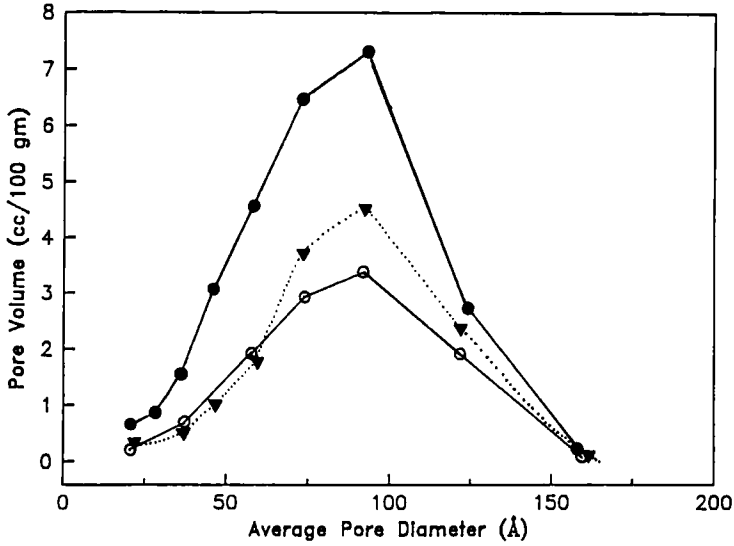

FIGURE 2. Plot showing the effect of surfactant sorption on Resolve C-18 pore volume as a function of average pore diameter: (●) untreated Resolve C-18; (▼) equilibrated with 6% Brij-35; and (⊙) equilibrated with 6% NaLS.

and others (25,26). The hysteresis loops for both materials (untreated and surfactant modified) were strikingly similar to loops previously reported for silica, with the loop shape indicative of a structure formed by compressing spheres together (11,12). Thus, it appears that the general pore shape of the parent C-18 packing is retained in the surfactant-modified material. That is, the surfactant coats out as a thick film on the interior capillary walls rather than completely filling the pores (11,12).

Figure 2 shows the pore volume as a function of average pore diameter for the unmodified Resolve C-18 reference material and for Resolve C-18 which had been equilibrated with solutions of 6% Brij-35 and 6% NaLS. These curves show that the curve shapes are all similar, with maximal contribution to the pore volume from pores with an average diameter of about 100 Å. Similar curve

shapes were obtained for the other surfactant/concentration combinations examined (11,12). The fact that the surfactant-modified phases exhibit decreased pore volumes yet retain their general pore size distribution profile supports the previous conclusion that the surfactant is adsorbed onto the C-18 packing as a thick continuous film, with the general pore structure and pore shapes of the original material retained (12).

Lastly, the effect of different primary alcohol additives in the micellar solution upon the amount of surfactant sorbed, S_{BET}, and cumulative pore volume of the C-18 material was determined. The results are summarized in Table 2. As can be seen, exposure of the C-18 packing material to 0.285 M NaLS alone resulted in approximately 150 mg of sorbed surfactant per gram of Resolve C-18. The addition of 5% methanol, ethanol, propanol, butanol, or pentanol to the 0.285 M NaLS resulted in a progressive decrease in the amount of sorbed surfactant as the alkyl group of

TABLE 2

Effects of Alcohol Modifiers on Stationary Phase Modification[a]

0.285 M NaLS Micellar Solution plus Indicated Alcohol Modifier	NaLS Sorbed (mg/gram Resolve C-18)	BET Surface Area (m^2/ gram Resolve C-18)	Cumulative Pore Volume (cc/gram)
None	146	59	0.135
5% MeOH	132	63	0.146
5% EtOH	128	68	0.153
5% 1-PrOH	104	80	0.176
5% 1-BuOH	101	88	0.198
5% 1-PeOH	83	97	0.213
Unmodified Resolve C-18	0	121	0.279

[a]Data adapted from References 11,12.

the alcohol modifier increased (11,12). The addition of 1-pentanol reduced the amount of sorbed NaLS by ca. 45%. Additionally, the BET surface area and cumulative pore volume increased significantly approaching the limiting values observed for the original unmodified C-18 material (Table 2). Thus, the addition of alcohol modifiers to micellar solutions clearly alters the characteristics of the surfactant modified stationary phase and decreases the amount of sorbed surfactant compared to that possible in their absence. Several other recent reports have concluded that other organic solvents such as THF and acetonitrile also seem to compete with the surfactant for adsorption on C-18 stationary phase materials (22,23). Taken together, these results indicate that the presence of appropriate organic additives can significantly decrease the amount of surfactant sorbed onto the stationary phase. The amount of surfactant desorbed by such additives is proportional to the additive concentration and increases as the hydrophobicity of the additive increases (12,23).

The fact that appreciable amounts of surfactant are adsorbed onto the C-18 stationary phase material and that additives can alter the extent of this coverage has important implications with respect to efficiency in MLC. We and others have previously noted that the origins of the generally poor chromatographic efficiency observed in MLC may be traced to the nature of the surfactant-coated stationary phase (10-16,20-23,27). Namely, the surfactant-modified C-18 stationary phase is quite different from the unmodified C-18 phase in terms of its carbon load, effective film thickness, viscosity, and fluidity of the ligand-surfactant modified surface, all of which adversely impact MLC efficiency (12,14). These altered stationary phase properties are expected to predominantly affect the last term of equation 4, which shows the various factors contributing to the theoretical plate height, H:

$$H = \frac{1}{(1/C_e d_p) + (1/(C_m d_m^2 u/D_m))} + \frac{C_d D_m}{u} + \frac{C_{sm} d_p^2 u}{D_m} + \frac{C_s d_f^2 u}{D_s} \quad \text{(eq. 4)}$$

where: C_e, C_m, C_d, C_{sm}, and C_s are the plate height coefficients due to Eddy diffusion, mobile-phase mass transfer, longitudinal diffusion, stagnant mobile-phase mass transfer, and stationary-phase mass transfer, respectively, with the other variables being the particle diameter, d_p; mobile phase velocity, u; solute diffusion coefficient in the mobile and stationary phases, D_m and D_s, respectively; and stationary phase film thickness, d_f (28). In MLC, the nature of the surfactant-modified stationary phase is such that the effective film thickness is increased and the solute diffusion coefficient in the modified phase significantly diminished, both of which lead to reduced efficiency (11,12,16,23). All of the alcohol and other additive effects observed in MLC using C-18 stationary phases can be rationalized in terms of their effect upon the surfactant-modified stationary phase and the last term in eq. 4 (14) *vide infra*.

Investigation of the Retention Mechanism in MLC using a Homologous Series.

The use of linear free energy relationships to study the retention mechanism in reversed-phase liquid chromatography has been reported by a number of workers. It can be shown that the retention of a homologous series of solutes is related to the carbon number for each solute in the series by the following relationship:

$$\log k' = n_c \log \alpha + \log \beta \qquad \text{(eq. 5)}$$

where n_c is the number of carbon atoms in the homolog, α is the non-specific selectivity of a methylene group, and β is the retention contribution from the functional group common to the series (29). In contrast to the typical linear relationship between log k' and n_c observed in RPLC using traditional hydro-organic mobile phases, we and others have noted that a linear relationship between k' and n_c seems to exist in MLC (11,13,30). This has been observed for Brij-35, Brij-22, NaLS, NaDC, CTAB, and CTAC as the micelle-forming surfactants using C-18 and C-8

stationary phases and n-alkylbenzenes, 2-alkylanthraquinones, or n-alkylphenones as the homologous series (11,13,15,30). One practical advantage to be gained from this linear k' vs. n_c relationship is that a greater number of solutes of the homologous series will be eluted per unit time in the isocratic mode in MLC compared to that of conventional RPLC (13).

Our investigations of the origins of this linear k' vs. n_c relationship in MLC have led to a better understanding of MLC in general, and of retention phenomena in particular (13). Our first objective was to apply the Armstrong-Nome approach to the determination of the different partition coefficients unique to the MLC separation of members of a homologous series and to examine the relationship between these respective partition coefficients and solute homolog number. In the context of that work, we had to develop a more accurate procedure for determining the chromatographic phase ratio, which is required in a plot of the MLC retention data according to eq. 3. Previously, the stationary phase volume, V_s, was taken to be the difference between the empty column volume and the packed column void volume (3,19,21, 31). This difference is clearly a poor estimate of V_s since it includes the entire volume occupied by the silica support rather than just the true stationary phase and hence gives an inflated value for V_s. Although use of this V_s for calculation of the phase ratio required in eq. 3 for determination of the MLC partition coefficients results in accurate values for P_{mw} (3,19), the values obtained for the P_{sw} and P_{sm} coefficients will be significantly in error.

The new procedure for determination of V_s and hence phase ratio which we have developed (eq. 1) requires measurement of the total weight of the packing in the column and the cumulative pore volumes (cm^3/gram) of the unbonded silica support material, CPV_s, and the bonded C-18 Resolve packing material, CPV_b, before or after exposure to the micelle-forming surfactant (13). This approach completely excludes any volumes associated with the base silica material since the stationary phase volume is assumed

to be that portion of the silica pore volume filled upon bonding the octadecylsilane (or other alkyl ligand) chains and, in the presence of micelles, by sorption of the surfactant to the C-18 chains. It is thought that the calculation of the phase ratio by this approach will be much more accurate and thus result in more meaningful quantitative determination of the P_{sw} and P_{sm} partition coefficients in MLC. In addition, this procedure should prove useful in conventional RPLC, in general, given that the same problems are encountered in such estimation of V_s as has been recently discussed (31).

Calculation of V_s by this new method revealed that the phase ratio is logarithmically related to the micellized surfactant concentration and mimics the surfactant - Resolve C-18 packing material adsorption profiles (11,13). Thus, strictly speaking, one cannot correctly assume that V_s and thus φ are constant over the entire surfactant range and use only one phase ratio value in the treatment of MLC data according to eq. 3, without risking significant error. This margin of error may be large for MLC experiments conducted at relatively low surfactant concentrations. In addition, it may not always be appropriate to consider the entire sorbed surfactant volume as part of the 'active' stationary phase in MLC and hence corrections taking into account actual solute solubilization sites may be required, particularly in the calculation of the P_{mw} values (13).

The plots of $1/k'$ vs. C_m according to equation 3 for members of an alkylbenzene homologous series eluted from the Resolve C-18 column with micellar Brij-35 were linear (correlation coefficient ≥ 0.998) (12,15). However, for all of the micellar mobile phases examined, such plots yielded apparent 'negative' intercepts for homolog members with $n > 3$ (Table 3). After expending considerable effort to determine whether or not this was attributable to procedural artifacts, it was concluded that, within experimental error, these negative intercepts are really approximately zero rather than actually negative. This merely reflects the great solute affinity for the micellar or surfact-

TABLE 3

Summary of Results of Regression Analyses for Elution of Alkylbenzenes with Micellar Brij-35 Mobile Phases[a]

Test Solute	P_{mw}[b]
Benzene	34.2
Toluene	95.9
Ethylbenzene	291
n-Propylbenzene	934
n-Butylbenzene	[c] (-0.00004)
Amylbenzene	[c] (-0.00016)
Phenylhexane	[c] (-0.00016)
Phenylheptane	[c] (-0.00006)

[a]Data taken from References 11,13. [b]Partition coefficient for distribution of the solute between the micelle pseudophase and bulk water of the mobile phase. Determined from the slope/intercept ratio of plots of the data according to eq. 3.

[c]P_{mw} could not be determined due to 'negative' intercepts (which are given in parenthesis). The standard error was ± 0.000075 (13).

ant coated (micellar-like) stationary phase compared to that of the bulk aqueous component of the mobile phase. That is, if P_{sw} for a solute is very large, then $1/P_{sw}$, which is the intercept of the plot of equation 3, would be expected to approach zero (13). As expected, solubility data indicated that these higher molecular weight homologs are virtually insoluble in water. This finding implies that in MLC, such very hydrophobic solutes can only be transported between the micelles in the mobile phase and the surfactant-modified stationary phase by a direct transfer process (P_{sm}) (Figure 3) (13).

In addition, it was observed that the plots of chromatographic selectivity as a function of homolog number exhibited a discontinuity with micellar mobile phases. That is, the selectivity factors were found to decrease with homolog number down

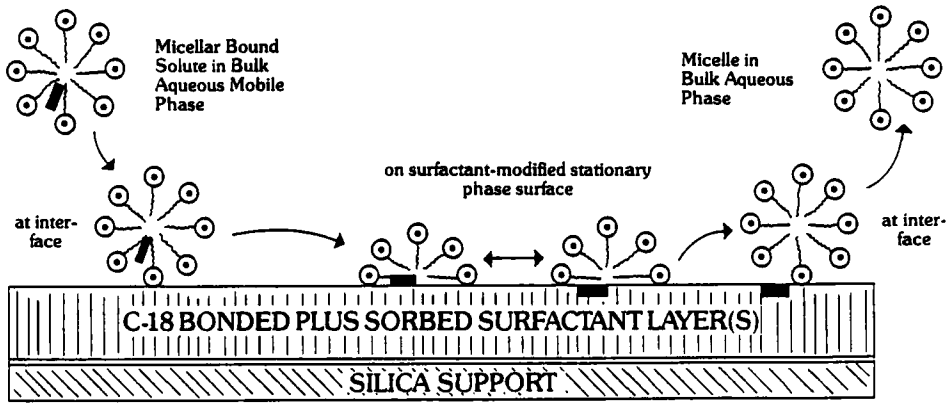

FIGURE 3. Artistic representation of the direct transfer process for distribution of a solute between the micelle pseudophase and the surfactant-modified (hemimicellar) C-18 stationary phase. (Reprinted with permission from Reference 13, American Chemical Society.)

to about n = 4 after which they leveled off to an approximate constant value at the solute water solubility limit of butylbenzene. In contrast, elution with a traditional hydroorganic mobile phase resulted in essentially constant selectivity factors for the alkylbenzene homologs studied (13). The observed discontinuity in such plots of methylene group selectivity vs. n_c for solutes eluted with the micellar mobile phases also argues for a possible subtle difference in the MLC retention process for water soluble as opposed to water insoluble members of the homologous series examined (11,13).

Although mentioned in theories of pseudophase liquid chromatography (3,19), the possibility of a direct transfer of a solute from the micelle in the mobile phase to the surfactant-coated stationary phase has been largely ignored. Our work indicates that the retention of insoluble or sparingly water soluble solutes is governed by the partition coefficient associated with such direct transport process. A modified form of the

Armstrong-Nome equation (eq. 6) can be derived which successfully accounts for the dependence between k' and C_m observed for elution of such solutes in MLC (13):

$$k' = P_{sm}(\varphi/\bar{v}C_m) \qquad \text{(eq. 6)}$$

In fact, by use of eq. 6, P_{sm} values determined at any given micellar mobile phase composition can be employed to predict solute retention for other mobile phase concentrations with little error over the entire alkylbenzene homologous series range (13).

Effect of Organic Modifiers in Micellar Liquid Chromatography.

The effect of different organic additives (i.e. alcohols, alkane diols, alkanes, alkylnitriles, and dipolar aprotic solvents) upon the solute retention, eluent strength, and chromatographic efficiency observed for elution of two neutral test solutes, benzene and 2-ethylanthraquinone, from a C-18 stationary phase with micellar NaLS, CTAC, NaDC, and Brij-35 mobile phases was determined. These results were contrasted to separations obtained using conventional methanol:water mobile phases (14,15). The test solutes were chosen in part due to the fact that benzene is relatively water soluble and 2-EtAQ is virtually water insoluble. Thus, these solutes represent two extremes with respect to the process by which the solute partitions between the micelle in the mobile phase and the surfactant-modified stationary phase, as just described. It should again be noted that the presence of additives can alter the micellar parameters as previously mentioned (Table 1) as well as the nature and properties of the micellar aggregate (14,15).

Additive Effects upon MLC Retention. Previously, the use of organic additives in MLC has been mentioned as a means to improve efficiency (32,33). However, little has been published concerning the use of organic modifiers to control solute retention in MLC (10). In general, our results indicate that the presence of alcohol, diol, dipolar aprotic solvents (DMSO, dioxane), and the alkylnitrile organic additives in either the NaLS or CTAC micellar mobile phases resulted in a diminution of the

capacity factor for the two test solutes (14,15). In contrast, the presence of alkane additives (i.e. pentane, hexane, cyclohexane) did not greatly alter the retention compared to that observed in their absence. Figure 4 illustrates the dependence of k' for 2-EtAQ as a function of the carbon homolog number of the added 1-alkanol modifier at several NaLS surfactant and alcohol concentration levels. The capacity factor was found to decrease as the carbon number (hydrophobicity) of the alcohol modifier was increased. The reduction in k' is more pronounced for micellar solutions containing greater amounts of the additive (compare the curves for 5 and 10% added alcohol modifier in NaLS in Figure 4). The alcohol modifier effects upon retention are attenuated as the surfactant concentration is increased (compare the 5% added alcohol curves in the presence of 0.285 and 0.475 M NaLS, Figure 4). In addition, the effect of the organic modifier upon the capacity factor of a solute is more dramatic the greater the hydrophobicity of the test solute (14). Thus, the reduction in k' observed upon addition of organic modifiers to micellar mobile phases depends not only upon the identity and concentration of the organic modifier, but also upon the surfactant concentration and the hydrophobicity of the test solute (14,15).

Although only briefly examined for a limited number of organic modifiers (i.e. propanol, butanols, pentanols), our results suggest that a linear relationship exists between the reciprocal of the capacity factor and the molar concentration of the additive for micellar mobile phases containing a fixed surfactant concentration (14). A replot of some literature data (6) (see Figure 5) for the elution of benzene from an ODS column using a 0.05 M NaLS micellar mobile phase with propanol as the modifier serves to illustrate this 1/k' vs. [modifier] relationship. Previously, Khaledi et al had reported a linear dependence between log k' and the volume fraction of 2-propanol in micellar mobile phases for elution of a homologous series of solutes (30). The relative effectiveness of an organic modifier in MLC appears to be directly related to its own ability to partition and bind to the mi-

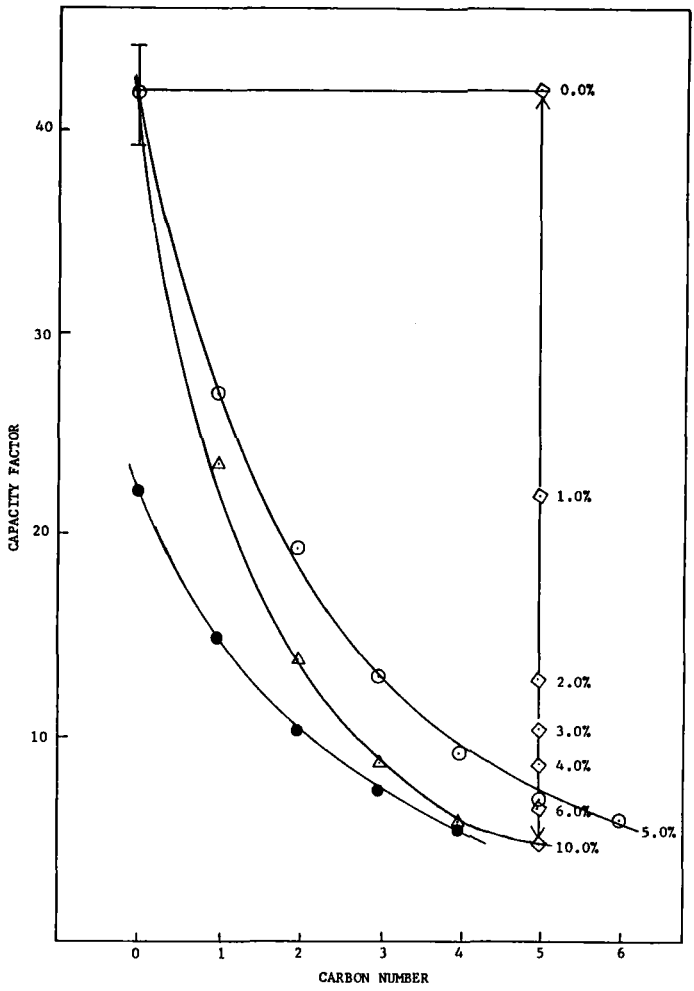

FIGURE 4. Dependence of the capacity factor of 2-ethylanthraquinone upon carbon number of the added 1-alkanol present at 5% (v/v) (⊙) and 10% (v/v) (△) in a 0.285 M NaLS micellar mobile phase or 5% (v/v) (●) in a 0.475 M NaLS micellar mobile phase. For 1-PeOH (carbon # = 5), the capacity factor as a function of added percentage 1-PeOH (v/v) in 0.285 M NaLS is also presented (◇). Conditions: 10 cm 5 μ C-18 column; temperature 23.5° C; and 1.00 mL/min flow rate except for the runs utilizing 5% 1-hexanol and 10% 1-pentanol which were run at a flow rate of 0.50 mL/min. (Reprinted with permission from Reference 14, American Chemical Society.)

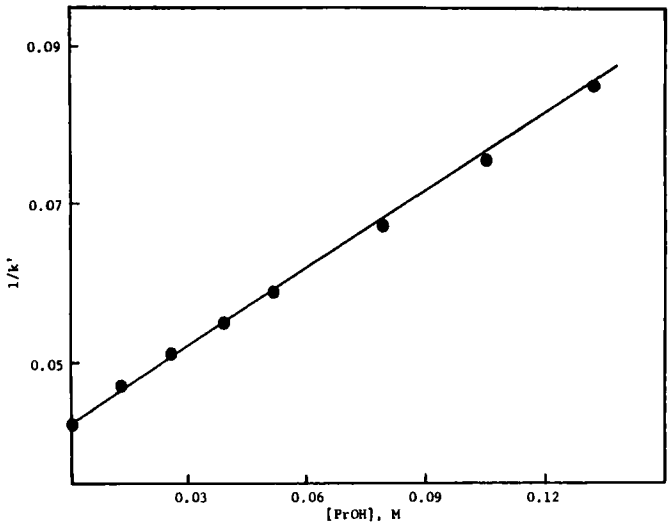

FIGURE 5. Linear relationship between retention and propanol modifier concentration in a 0.05 M NaLS micellar mobile phase for benzene as the test solute. Conditions: 4.6 x 250 mm Ultrasphere ODS column, 40° C. Data taken from Reference 6 (Table 3).

cellar pseudophase (14). That is, the better the organic modifier binds to the micellar assembly, the greater is its ability to alter the retention of neutral solutes in MLC on C-18 phases.

The organic additive effects upon MLC retention can be rationalized in terms of the change in the nature of the surfactant-modified stationary phase occuring in the presence of the additive and in terms of the enhanced solute solubility in the organic modified micellar assembly. In terms of the P_{sw} and P_{mw} pseudophase partition coefficients, a limited study indicates that the addition of alcohol or diol modifiers to the micellar system results in a shift in the distribution of the solute from both the micellar and surfactant-modified stationary phase to the bulk solvent in the mobile phase (14).

In terms of practical consequences, micellar mobile phases allow for the use of organic additives in an aqueous solution at

molar concentrations well above their normal solubility limit in water alone. For example, the water solubilities of pentanol and pentane are ca. 0.30 and 0.00053 M, respectively; however, in 0.285 M NaLS micellar media, their solubility increases to ca. 0.94 and 0.096 M, respectively. More importantly, the use of organic modifiers in MLC can result in dramatic reductions in the elution times of solutes compared to that observed with the pure aqueous micellar mobile phase alone (14). For instance, the capacity factor of 2-EtAQ is reduced 54% and 57% by the presence of 5% 1-pentanol in micellar 0.15 M CTAC and 6% Brij-35 mobile phases, respectively, compared to that in the absence of the modifier (15). In addition, as can be seen from the right-hand line in Figure 4, the capacity factor for 2-EtAQ eluted with a 0.285 M NaLS mobile phase using a C-18 stationary phase was ca. 42 while that obtained for the same micellar mobile phase containing 5% 1-pentanol was 7.4. Thus, relatively small amounts of an organic modifier can greatly affect the eluent strength of micellar mobile phases and solute retention in MLC. This is particularly important when attempting to separate very hydrophobic components, as polycyclic aromatic hydrocarbons, in MLC (34). Additionally, our work suggests that organic modifier gradients can be successfully employed in MLC. However, in gradient elution MLC work, column re-equilibration would be required in view of the fact that the amount of surfactant coverage is altered by the organic additive present (Table 2).

Organic Modifier Effects upon Chromatographic Efficiency in MLC. A major problem of MLC is the reduced chromatographic efficiency observed compared to that possible with traditional hydro-organic mobile phase systems in RPLC (6,10,14,32-34). The magnitude of the problem becomes quite evident if one compares the efficiency data for elution of the test solutes with aqueous NaLS micelles in the absence of any organic modifiers (Table 4) to that of a reference aqueous methanolic mobile phase system. For example, comparison of the efficiency data for benzene at comparable

k' values reveals that there is a 75% reduction in the chromatographic efficiency on going from a mobile phase consisting of 50:50 MeOH:H_2O (N=6010) to 0.285 M NaLS (N=1530). The effect is even more dramatic for the more hydrophobic test solute, 2-EtAQ. For this solute, a 99% reduction in efficiency is observed upon changing from 60:40 MeOH:H_2O (N=7040) to 0.285 M NaLS (N=50) (14). Thus, this data illustrates the main drawback with MLC and shows that this chromatographic efficiency problem becomes progressively worse as the hydrophobicity of the test solute increases (14,32).

The effect of the presence of 25 organic additives to a NaLS micellar mobile phase on the chromatographic efficiency of the two selected test solutes was determined (14). The presence of all additives examined resulted in improved efficiency for elution of benzene. Concomitant with the enhanced chromatographic efficiency was an improvement in the peak symmetry. Some of the data for alcohol and alkane modifiers are summarized in Table 4. These results show that maximum efficiency can be obtained by adding small amounts of C-4 or C-5 alcohols or pentane as the organic modifier. This is especially true for elution of 'water-soluble' solutes using ionic micellar mobile phases (such as NaLS or CTAC) in MLC. With these additives, one can obtain 60-80% of the efficiency that is observed with a conventional 50% methanol hydroorganic mobile phase for this test solute. Thus, use of butanol, pentanol, or pentane as modifiers in MLC should be considered in lieu of 1-propanol which had previously been recommended as 'best' (33).

Except for alkane modifiers, the same trends as mentioned above concerning the effect of organic additives in MLC, were reached based upon examination of the more hydrophobic, water-insoluble test solute, 2-EtAQ. The use of C-4 to C-6 alcohol additives with NaLS resulted in a 24-to 28-fold improvement in the efficiency for the 2-EtAQ peak compared to that in their absence. In CTAC, the improvement ranged from 3-to 5-fold. However, even with these improvements, the plate counts observed with these modifiers were only ca. 22% of that observed for

TABLE 4

Effect of Some Organic Modifiers upon the Chromatographic Efficiency Observed for Benzene and 2-Ethylanthraquinone as Test Solutes on a C-18 Column using Different Micellar Mobile Phases[a]

Organic Modifier (% v/v)	Efficiency (N) for Indicated Test Solutes in the Specified Micellar Mobile Phase Systems:[b]					
	0.285 M NaLS:		0.15 M CTAC:		6% Brij-35:	
	Benzene	2-EtAQ	Benzene	2-EtAQ	Benzene	2-EtAQ
None	1530	50	3740	490	2910	1250
5% MeOH	2070	58	3850	660	3220	1490
5% EtOH	2600	110	----	---	----	----
5% 1-PrOH	3110	320	4530	1200	3110	1400
5% 1-BuOH	3420	730	----	---	3020	1520
5% 1-PeOH	3570	950	----	---	3080	1470
Sat'd Pentane	3920	23	5190	440	3800	1340

[a]Data taken from References 14,15. [b]Conditions: 10 cm 5 μ C-18 column, flow rate 1.00 mL/min, 23.5° C. The additives were present at a concentration of 5% (v/v) in the indicated micellar mobile phases except that the micellar solutions were saturated with the alkane additive, pentane. Direct comparison of plate counts between the three micellar mobile phases should not be made since the data was obtained on three different C-18 columns and the capacity factors are different. By use of a methanol:water reference mobile phase system, these variables can be factored out and direct comparisons made as will be detailed elsewhere (15).

elution of this test solute with a conventional 70-80% methanol hydroorganic mobile phase reference system. Whereas alkane additives enhanced the efficiency of the benzene peak, their presence as modifiers in ionic NaLS or CTAC micellar mobile phases essentially had no effect on the efficiency compared to that achieved in their absence (refer to pentane data, Table 4). Consequently, the use of alkane additives in MLC is recommended only for the elution of relatively water soluble test components. For very hydrophobic, sparingly water soluble solutes, the use of amyl alcohol as the modifier is recommended. In addition, as previously recommended by Dorsey et al (6,33), the use of elevated temperature (40° C) in MLC along with the 1-pentanol modifier is required in order to achieve MLC efficiencies comparable to those of conventional RPLC for the separation of very hydrophobic solutes (14).

We had previously noted that the addition of alcohol modifiers did not improve the efficiency observed in MLC when using nonionic surfactant mobile phase systems (16). As can be seen from the HETP vs. linear velocity curves obtained for elution of benzene with 15:85 EtOH:H_2O, 6% Brij-35 micellar, and 6% Brij-35 containing 15% EtOH modifier mobile phase systems (Figure 6), the addition of the alcohol modifier actually slightly decreased the the efficiency (16). The efficiency data presented in Table 4 for elution of the two test solutes using nonionic Brij-35, with and without modifiers, reveals that the presence of these different alcohol modifiers does not appreciably alter the efficiency compared to that observed in their absence (15). Thus, in contrast to some literature reports (6,7,32,35) which imply that addition of alcohol modifiers significantly improves efficiency in nonionic micellar LC, our data (Table 4) show that in fact no meaningful improvements occur. The problem with the previous work was that the temperature effect was not separated from the alcohol modifier effect (refer to Table III, reference 35). That is, it was the increased temperature which caused the improved efficiency with the Brij-35 micellar mobile phase and not the

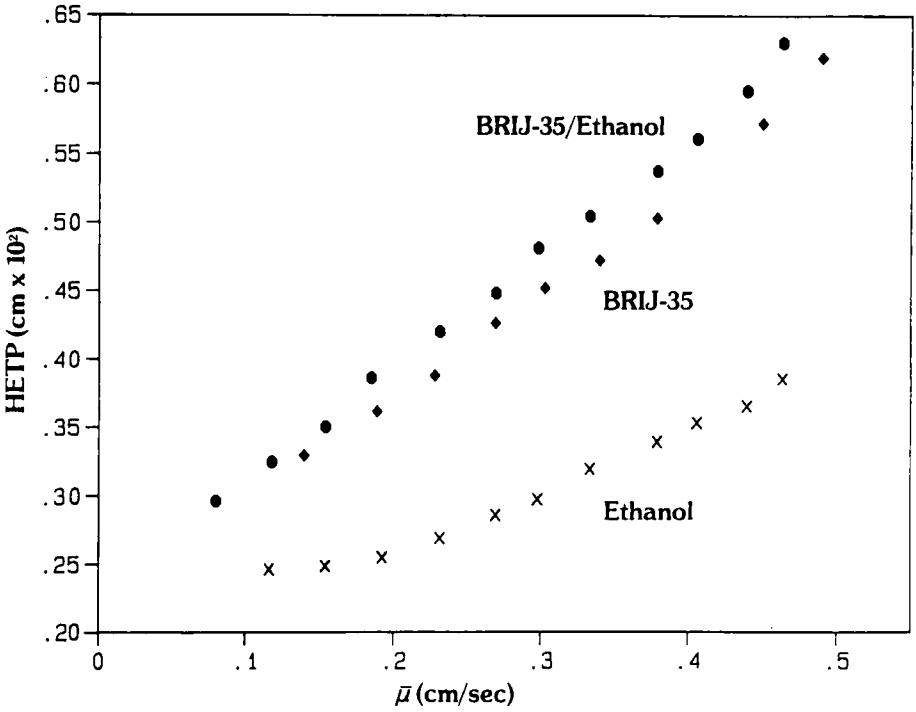

FIGURE 6. Relationship of the theoretical plate height, HETP, to mobile phase linear velocity, $\bar{\mu}$, for benzene as test solute on a Radical-PAK C-18 column using either (x) 15:85 (v/v) EtOH:H_2O; (◆) aqueous 6% Brij-35; or (●) 6% Brij-35 in a 15:85 (v/v) EtOH:H_2O mixture as the mobile phase. The capacity factors for benzene in these three mobile phase systems were 52, 27.4, and 20, respectively. (Reprinted with permission from Reference 16, American Chemical Society.)

presence of the 1-PrOH modifier. Thus, unless one wants to utilize alcohol modifiers for retention control as discussed in the previous section, it is not necessary to use such additives in nonionic MLC as no meaningful efficiency effects occur (15).

It should also be noted that the use of alkane additives (pentane, hexane) in nonionic micellar mobile phases does slightly improve chromatographic efficiency as was previously noted for

the ionic NaLS and CTAC micellar mobile phases (Table 4) for the elution of water 'soluble' components from C-18 stationary phases. Also, examination of the data in the Table suggests that the use of nonionic micellar mobile phased in the MLC separation of very hydrophobic solutes results in better inherent chromatographic efficiency compared to the use of charged, ionic micelles. In terms of the additive effects, it appears that the use of alkane modifiers results in improved MLC efficiency for elution of relatively water soluble components in both nonionic and ionic micellar mobile phase systems. For sparingly soluble solutes, the use of medium chain length alcohol modifiers (such as pentanol) appear most useful if ionic micellar mobile phases are utilized in MLC while such additives do not alter the efficiency observed when using nonionic micellar phases. It should be stressed that the above general guidelines apply only to the separation of neutral test solutes on a C-18 stationary phase with the micellar systems mentioned.

Chromatographic efficiency studies were also conducted in which additive concentrations were varied at a fixed surfactant concentration and in which the surfactant concentration was varied at a fixed modifier concentration. Table 5 presents some representative data for acetonitrile as modifier and CTAC as the micellar mobile phase on a C-18 stationary phase. As can be seen, increases in CTAC surfactant concentration at a fixed acetonitrile modifier concentration in the micellar mobile phase resulted in a progressive decrease in efficiency for both test solutes. This is the same trend as had been noted in the literature using micellar mobile phases in the absence of any additives (32). On the other hand, increases in the organic modifier concentration at a fixed CTAC concentration (Table 5) resulted in an increase in efficiency for both test solutes (15). These results are perhaps best illustrated by plotting the data as plate counts vs. the organic modifier to surfactant concentration ratio in the micellar mobile phase (Figure 7). When

TABLE 5

Summary of the Effects of Variation of the Acetonitrile Modifier or CTAC Surfactant Concentration on the Chromatographic Efficiency Observed for Benzene and 2-Ethylanthraquinone as Test Solutes[a]

Mobile Phase Composition	Efficiency	
	Benzene	2-EtAQ
Variation of CTAC surfactant concentration at a fixed 4.0% (v/v) acetonitrile modifier concentration level:		
[CTAC] = 0.05 M	6560	1550
0.10 M	5360	1230
0.15 M	4070	1000
0.20 M	3440	880
Variation of acetonitrile modifier concentration in a CTAC micellar mobile phase containing a [CTAC] = 0.15 M:		
[Acetonitrile] = 0.00%	3310	470
2.0 %	3910	770
4.0 %	4070	1000
6.0 %	4230	1240
8.0 %	4880	1480
10.0 %	5390	1780

[a]Data taken from Reference 15. Conditions are as mentioned in the caption to Figure 7.

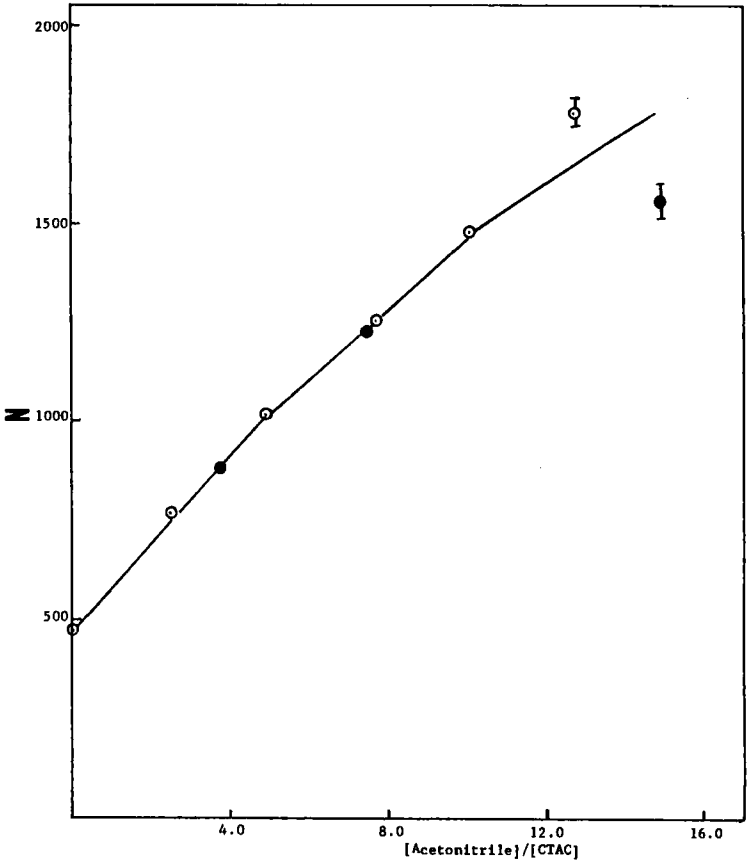

FIGURE 7. Plot of the plate counts vs. the ratio of acetonitrile modifier to CTAC concentration in the micellar mobile phase for elution of 2-EtAQ as the test solute. This graph is composed of data obtained from two types of experiments; i.e. variation of the acetonitrile concentration at a fixed 0.15 M CTAC concentration (o) and variation of the CTAC concentration at a fixed 4.0% acetonitrile concentration (●). Conditions: 10 cm 5 μ C-18 column, flow rate 1.0 mL/min, temperature 23.5° C.

viewed in this manner, it becomes abundantly clear that it is this modifier/surfactant concentration ratio which controls the efficiency observed in MLC with ionic micellar mobile phases. The higher this ratio, the better is the observed MLC efficiency and solute peak shape (14,15). This same general type of behavior was also observed for these two test solutes with other combinations of organic modifiers in the charged anionic NaLS or cationic CTAC micellar mobile phase systems examined (15). Of course, there is a practical limit as to how much modifier can be added to a given surfactant micellar solution since increases in the solution viscosity can occur as well as eventual formation of microemulsion systems with the continued addition of such organic modifiers to aqueous micellar media (10,14,36).

Lastly, several paragraphs will be devoted to a discussion of the potential origins of the efficiency effects noted with these two test solutes and the different micellar media examined. Previously, Dorsey et al have attributed the reduced efficiency in MLC to poor wetting of the stationary phase which slows mass transfer across the interface of two highly dissimilar phases (6, 7,33). Cline Love and co-workers concluded that the diminished efficiency was due to mass transfer problems stemming from the slow solute exit rates from the micelle in the mobile phase and slow desorption of the solute from the surfactant-modified stationary phase (32). This is the exact situation that one has for very hydrophobic solutes which require the direct transfer partitioning mode (13,14). As mentioned earlier, we and other have reported that the poor MLC efficiency predominantly stems from the nature of the surfactant-modified stationary phase and the poor mass transfer to, in, or from that phase (10-16,23). In fact, almost all of the types of additive effects observed upon chromatographic efficiency in our work can be rationalized in terms of their impact upon the surfactant-modified stationary phase and the last term in eq. 4.

Analysis of our data from efficiency studies using various organic modifiers in MLC has led to several insights as to their

role in efficiency improvement. First, a comparison of the relative effect of the different alcohol additives in the NaLS micellar mobile phase upon efficiency (Table 4) parallels their ability to desorb NaLS surfactant molecules from the C-18 bonded stationary phase (Table 2). In addition to reducing the carbon loading and film thickness, d_f, the addition of these alcohols is also expected to influence the fluidity/rigidity of the surfactant aggregate/C-18 bonded ligand structure on the stationary phase just as their presence reportedly alters the fluidity of the micellar aggregate structure (14,23,36). For instance, upon addition of increasing amounts of amyl alcohol to NaLS micellar solutions, the microviscosity of the interior of NaLS micelles, estimated to be 21 cP in the absence of the additive, was reduced to ca. 5 cP in the presence of 1 M modifier (36). One might reasonably expect similar alcohol effects upon the fluidity of the surfactant structure on the stationary phase which may improve efficiency since the solute diffusion coefficient, D_s, ought to increase as the microviscosity of the phase decreases.

Similarly, the observation that chromatographic efficiency improves with increasing organic modifier to surfactant concentration ratios (Figure 7) probably reflects the fact that the greater the concentration of the organic modifier, the greater will be the amount of surfactant desorbed from the stationary phase (lower carbon loading, smaller effective film thickness). In support of this interpretation, recent reports have demonstrated that increases in the organic modifier concentration in micellar solutions resulted in an almost linear decrease in the amount of surfactant desorbed from C-18 stationary phase materials (23,37). In addition, the higher level of modifier may also lead to increased fluidity of the organic additive modified surfactant-coated stationary phase as just described.

At a fixed modifier concentration, the efficiency was observed to decrease as the surfactant concentration in the mobile

phase was increased (Figure 7). One might be tempted to conclude that this is the result of the increased mobile phase viscosity observed with increased surfactant concentration which would impede solute mass transfer in the mobile phase. However, this is probably only a relatively minor factor since it was also noted that addition of increasing amounts of 1-PeOH (from 0 to 6%) increased the efficiency of both test solutes eluted with a 0.285 M NaLS micellar mobile phase (N increased from 1530 to 3640 and 50 to 1260 for benzene and 2-EtAQ, respectively) in spite of the fact that the relative microviscosity of the micellar mobile phase had increased from 1.65 to 2.75 due to the added alcohol (14). As stated in the previous paragraphs, it is thought that the additive to surfactant concentration ratio is the dominant factor influencing chromatographic efficiency since this ratio dictates the amount of surfactant coverage on the stationary phase and fluidity of the modified stationary phase. In agreement, results from recent diffusion studies also lends support to this argument (23,27).

Depending upon the test solute monitored, the presence of alkane additives had different effects on the MLC efficiency observed with ionic micellar mobile phases. That is, their presence in the mobile phase improved the efficiency for the relatively water soluble benzene test solute whereas they had essentially no effect upon the efficiency for the virtually water insoluble 2-EtAQ solute compared to that achieved in their absence (Table 4). It is thought that this dramatic difference in behavior reflects the fact that these two solutes undergo different transport modes with respect to their partitioning between the mobile and stationary phases. As mentioned in the homologous series study, our results show that water insoluble solutes, such as 2-EtAQ, can only partition between the micelle in the mobile phase and the surfactant-coated stationary phase via a direct transfer process (Figure 3). Consequently, for 2-EtAQ to desorb/exit the stationary phase or micelle in the

mobile phase, both of which it has a great affinity for, requires a merging of the ionic micelle with the surfactant-modified stationary phase. In the case of ionic micelles, both the ionic headgroup of the micelle in the mobile phase and the surface of the ionic surfactant-coated stationary phase will be similarly charged. Thus, an electrostatic repulsive barrier to the direct merger of the micellar entity with the surfactant-coated stationary phase will exist and impede solute mass transfer across this interface. The greater the fraction of partitioning which must occur via this direct transfer mode, the poorer will be the observed chromatographic efficiency. In contrast, water soluble solutes which do not have to undergo such transfer process exhibit enhanced efficiency due to the fact that alkane additives are even more effective than alcohols at desorbing surfactant from the surfactant-coated C-18 stationary phase (13-15).

In view of the above discussion, it should be noted that another potential reason that alcohols improve the efficiency in MLC with ionic surfactant micelles may be due to the fact that their presence can reduce the net electrical charge density of the ionic micellar surface (38). Thus for very hydrophobic test solutes, this would be expected to improve the mass transfer to/from the stationary phase/micelle since the extent of the electrostatic repulsive barrier to a direct transfer mode would be somewhat diminished. The literature reports that the presence of alkane additives does not affect the surface charge density, hence these type additives do not improve efficiency for very hydrophobic solutes despite the fact that they reduce the extent of surfactant coverage of the stationary phase (14,15,23). In addition, it has been reported that alcohol-modified ionic surfactant micelles are capable of collisions with one another which results in intermicellar transfer of solute molecules (36,38).

Lastly, the arguments employed in the previous paragraphs can be utilized to rationalize the fact that alcohol additives

are not effective in enhancing the efficiency in MLC with nonionic micellar mobile phases. Nonionic micellar surfactants are long chain tensioactive alcohols themselves and our preliminary data indicate that C-1 - C-5 alcohols are not very effective in desorbing these nonionic surfactants (high molecular weight alcohols) from the surfactant-modified C-18 stationary phase (15). Such nonionic surfactants are also not charged and therefore there is no electrostatic charge barrier encountered in a direct transfer process envisioned for water insoluble solutes. The efficiency achieved in chromatographing very hydrophobic test solutes with nonionic Brij-35 or Brij-22 micellar mobile phases is thus better than that which can be obtained with any ionic micelles. It is thought that alkane additives in nonionic MLC can further improve the efficiency observed for benzene, the relatively water soluble solute, because alkanes can desorb nonionic surfactants from the modified stationary phase (15).

Further work aimed at direct determination of the solute diffusion coefficient in the stationary phase, effective film thickness, and degree of surfactant coverage in the presence of different organic additive/surfactant micellar combinations and the temperature dependence of these parameters is in progress and the results should help to shed additional light on the nature of efficiency effects in MLC (15).

Preliminary Characterization of New Types of Surfactants for Use in Micellar Liquid Chromatography.

We have evaluated several new types of surfactant molecules, such as bile salts, ionenes, and ionic alkyltrimethylammonium halide surfactants which contain a hydroxyl group near the charged cationic headgroup, as potential micellar mobile phases in MLC. Bile salts, such as NaDC, are another class of surfactant-like molecules that can aggregate in aqueous media to form micellar assemblies. The term bile salt covers the several carboxylate derivatives of cholic acid which differ in the number and position of the hydroxy substituents. They differ from the typical long-

chain alkyl normal micelle-forming surfactants previously employed in MLC in that they have a rigid cholesterol-like steroidal ring structure and possess a hydrophobic and hydrophilic face (10). They also exhibit a different association behavior and aggregate structure compared to that of conventional normal micelles. That is, bile salt aggregation is viewed as consisting of the step-wise formation of small primary bile salt micelles consisting of 2-8 monomers held together by hydrophobic interactions. At higher bile salt concentrations, larger, secondary bile salt aggregates are thought to form due to intermolecular hydrogen bonding between the bile salt's hydroxyl groups. Sodium deoxycholate is reported to undergo this type of aggregation behavior (10). In addition, bile salt molecules are chiral and can form chiral aggregates possessing helical structures. Investigation of such micellar media in MLC is important in at least two respects. First, since chiral micelles form, one can potentially utilize such micellar mobile phases in optical separations. Secondly, there are many solubilization procedures reported in the clinical and biological literature which utilize bile salts to extract desired constituents from cells, protein materials, etc. In such solubilization and extraction procedures, it is often necessary to remove the bile salt prior to further chromatographic separation and quantitation of the desired analyte. Thus, if such bile salt molecules could also function as mobile phases in MLC, then this would obviate the need to remove them after the solubilization/extraction step and ensure for compatibility between the solubilization/extraction 'solvent' and the chromatographic mobile phase.

Due to these reasons, we have conducted a preliminary investigation in order to determine if bile salt surfactants, such as NaDC, can function as effective micellar mobile phases in MLC. With respect to elution of solutes, such as benzene, pyrene, and 2-EtAQ, with NaDC micellar mobile phases, we found that the capacity factors decreased as the NaDC concentration in

the mobile phase increased as expected based on the pseudophase MLC theory. However, attempts to apply the Armstrong-Nome treatment (i.e. eq. 3) resulted in curved rather than linear plots of 1/k' vs. [NaDC]. This was expected however in view of the fact that NaDC undergoes a step-wise association process (i.e. changing CMC and N as a function of NaDC concentration) which means that the C_m term of eq. 3 must take this into account. The problem with this is that such data is not readily available in the literature. Consequently, if one does not account for this type of association, curved rather than linear plots result. With respect to additive effects upon retention and efficiency, essentially the same general trends as previously noted for the anionic surfactant NaLS were found to also hold for the anionic NaDC. Thus, use of 1-pentanol, 1-hexanol, or 1-heptanol as an organic modifier is recommended for use with NaDC bile salt micellar mobile phases (15).

In terms of separations achieved, the use of NaDC micellar mobile phases allowed for the separation of a wide range of different solute test mixes, including polycyclic aromatic hydrocarbons (34), quinones, vitamin K's, cis/trans isomers, positional isomeric methylindoles, and steroids among others. Most important, it appears that use of this mobile phase allows for the optical resolution of binaphthol and related isomers. For example, Figure 8 shows the partial separation of the enantiomers of S-(+)- and R(-)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate with a 80 mM NaDC micellar mobile phase containing 4% added 1-PeOH (15). Similar separations were possible with this mobile phase for enantiomers possessing C-2 symmetry. Since such enantiomeric compounds are now widely employed in organic synthetic schemes for enantioselective synthesis, the use of bile salt micellar mobile phases may prove very useful for optical resolutions required in such work.

In addition to bile salts, we are also investigating whether polymeric ionene surfactants, which can form intramolecular,

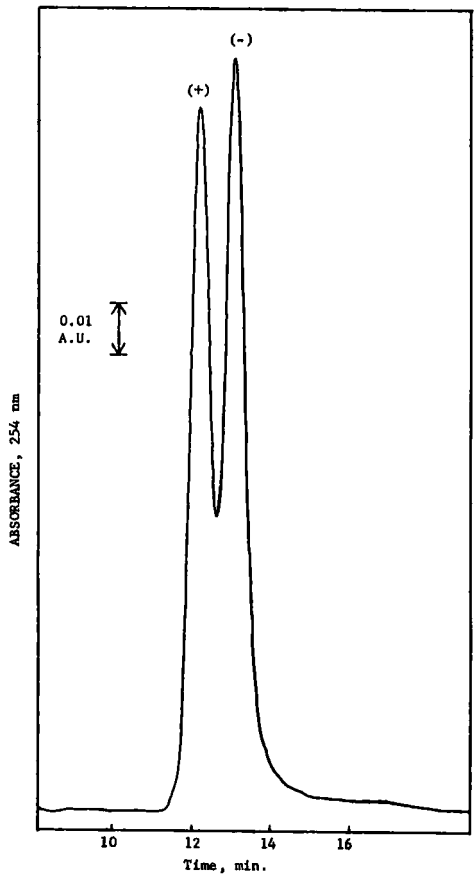

FIGURE 8. Chromatograph trace which shows the partial resolution of the S-(+)- and R-(-)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate enantiomers with a 0.080 M NaDC micellar mobile phase containing 4.0% 1-pentanol as modifier on a 25-cm C-18 column (flow rate = 0.4 mL/min).

micellar-like aggregates (39), can function not only as 'micellar' mobile phases, but also as immobilized stationary phases in MLC. The preliminary results have been encouraging and will be presented in greater detail elsewhere (15).

ACKNOWLEDGMENTS

The authors thank their co-workers, whose names appear in the references listed, for their dedicated and enthusiastic work. We thank the R. J. Reynolds Tobacco Company, The Petroleum Research Fund (Type AC Grant), and Wake Forest University for financial support of this research project. Portions of this paper were presented at the Symposium on Surfactant-Based Separation Processes at the 196th National Meeting of the American Chemical Society, September 25, 1988, Los Angeles, CA. and 27th Eastern Analytical Symposium, October 6, 1988, New York, NY.

REFERENCES

1. Armstrong, D. W.; Terrill, R. G., Thin-Layer Separation of Pesticides, Decachlorobiphenyl and Nucleosides with Micellar Solutions, Anal. Chem., 51, 2160, 1979.

2. Armstrong, D. W.; Henry, S. J., Use of an Aqueous Micellar Mobile Phase for Separation of Phenols and Polynuclear Aromatic Hydrocarbons via HPLC, J. Liq. Chromatogr., 3, 895, 1980.

3. Armstrong, D. W., Application of Pseudophase Liquid Chromatography (PLC), Amer. Lab., 13, 14, 1981.

4. Armstrong, D. W., Micelles in Separation: A Practical and Theoretical Review, Sep. Purif. Methods, 14, 213, 1985.

5. Liu, H.; Ling, J.; Yan, X., Pseudophase Liquid Chromatography and its Applications, Zhejiang Gongxueyuan Xuebao, 29, 39, 1985.

6. Dorsey, J. G., Micellar Liquid Chromatography, Adv. Chromatogr., 27, 167, 1987.

7. Dorsey, J. G., Micellar Mobile Phases for Liquid Chromatography: Academic Research Curiosity or Useful Alternatives?, Chromatogr., 2, 13, 1987.

8. Khaledi, M. G., Micellar Reversed Phase Liquid Chromatography, BioChromatogr., 3, 20, 1988.

9. Khaledi, M. G., Bioanalytical Capabilities of Micellar Liquid Chromatography, Trends Anal. Chem., 7, 293, 1988.

10. Hinze, W. L., Organized Surfactant Assemblies in Separation Science, In Ordered Media in Chemical Separations, Hinze, W. L.; Armstrong, D. W., Eds., American Chemical Society, Washington, D.C., 1987, pp. 2-82.

11. Borgerding, M. F., Studies of the Use of Nonionic Surfactants as the Organic Modifier Component of the Mobile Phase in Reversed-Phase Liquid Chromatography, Ph.D. Dissertation, Wake Forest University, Winston-Salem, NC, 1988.

12. Borgerding, M.F.; Hinze, W. L.; Stafford, L. D.; Fulp, G. W.; Hamlin, W. C., Investigations of Stationary Phase Modification by the Mobile Phase Surfactant in Micellar Liquid Chromatography, Anal. Chem., 61, submitted, 1988.

13. Borgerding, M. F.; Ouina, F. H.; Hinze, W. L.; Bowermaster, J.; McNair, H. M., Investigation of the Retention Mechanism in Nonionic Micellar Liquid Chromatography Using an Alkylbenzene Homologous Series, Anal. Chem., 60, 2520, 1988.

14. Hinze, W. L.; Fu, Z. S.; Williams, R. W.; Sadek, F. S.; Berthod, A., Effect of Organic Additives in Micellar Liquid Chromatography with Sodium Dodecylsulfate (NaLS) Micellar Mobile and C-18 Stationary Phases, Anal. Chem., 61, submitted, 1989.

15. Williams, R. W.; Fu, Z. S.; Borgerding, M. F.; Ouina, F. H.; Hinze, W. L., unpublished results.

16. Borgerding, M. F.; Hinze, W. L., Characterization and Evaluation of the Use of Nonionic Polyoxyethylene(23)dodecanol Micellar Mobile Phases in Reversed-Phase High-Performance Liquid Chromatography, Anal. Chem., 57, 2183, 1985.

17. Barrett, E. P.; Joyner, L. G.; Halenda, P. P., The Determination of Pore Volume and Area Distribution in Porous Substances. I. Computations from Nitrogen Isotherms, J. Amer. Chem. Soc., 73, 373, 1951.

18. Foley, J. P.; Dorsey, J. G., Equations for Calculation of Chromatographic Figures of Merit for Ideal and Skewed Peaks, Anal. Chem., 55, 730, 1983.

19. Armstrong, D. W.; Nome, F., Partitioning Behavior of Solutes Eluted with Micellar Mobile Phases in Liquid Chromatography, Anal. Chem., 53, 1662, 1981.

20. Berthod, A.; Girard, I.; Gonnet, C., Micellar Liquid Chromatography. Adsorption Isotherms of Two Ionic Surfactants on Five Stationary Phases, Anal. Chem., 58, 1356, 1986.

21. Berthod, A.; Girard, I.; Gonnet, C., Additive Effects on Surfactant Adsorption and Ionic Solute Retention in Micellar Liquid Chromatography, Anal. Chem., 58, 1362, 1986.

22. Berthod, A.; Girard, I.; Gonnet, C., Stationary Phase in Micellar Liquid Chromatography: Surfactant Adsorption and Interaction with Ionic Solutes, In Ordered Media in Chemical Separations, Hinze, W. L.; Armstrong, Eds., American Chemical Society, Washington, D.C., 1987, p. 130.

23. Berthod, A.; Roussel, A., The Role of the Stationary Phase in Micellar Liquid Chromatography: Adsorption and Efficiency, J. Chromatogr., submitted, 1988.

24. Sasaki, T.; Yasuoka, M.; Suzuki, H., Studies of the Measurement of the Intermicellar Concentration of Surfactants by Gel Filtration, Bull. Chem. Soc. Japan, 50, 2538, 1977.

25. deBoer, J. H., The Shape of Capillaries, In The Structure and Properties of Porous Materials, Everett, D. H.; Stone, F. S., Eds., Butterworths Scientific Publications, London, 1958.

26. Gregg, S. R.; Sing, K. S. W., Adsorption, Surface Area and Porosity, Academic Press, New York, 1967.

27. Armstrong, D. W.; Ward, T. J.; Berthod, A., Micellar Effects on Molecular Diffusion: Theoretical and Chromatographic Considerations, Anal. Chem., 58, 579, 1986.

28. Synder, L. R.; Kirkland, J. J., Introduction to Modern Liquid Chromatography, John Wiley & Sons, Inc., New York, 1979, Ch. 5.

29. Jandera, P., Reversed-Phase Liquid Chromatography of Homologous Series. A General Method for Prediction of Retention, J. Chromatogr., 314, 13, 1984.

30. Khaledi, M. G.; Peuler, E.; NgehNgwainbi, J., Retention Behavior of Homologous Series in Reversed Phase Liquid Chromatography using Micellar, Hydroorganic, and Hybrid Mobile Phases, Anal. Chem., 59, 2738, 1987.

31. Sentell, K. B.; Dorsey, J. G., On the Calculation of the Stationary Phase Volume in Reversed Phase Chromatography, J. Liq. Chromatogr., 11, 1875, 1988.

32. Yarmchuk, P.; Weinberger, R.; Hirsch, R. F.; Cline Love, L. J., Effects of Restricted Mass Transfer on the Efficiency of Micellar Chromatography, J. Chromatogr., 283, 47, 1984.

33. Dorsey, J. G.; DeEchegaray, M. T.; Landy, J. S., Efficiency Enhancement in Micellar Liquid Chromatography, Anal. Chem., 55, 924, 1983.

34. Hinze, W. L.; Singh, H. N.; Fu, Z. S.; Williams, R. W.; Kippenberger, D. J.; Morris, M. D.; Sadek, F. S., Micelle-Mediated Methodologies for the Preconcentration and Separation

of Polycyclic Aromatic Compounds, In Chemical Analysis of Polycyclic Aromatic Compounds, Vo-Dinh, T., Ed., John Wiley & Sons, New York, 1989, Ch. 5.

35. Cline Love, L. J.; Habarta, J. G.; Dorsey, J. G., The Micelle-Analytical Chemistry Interface, Anal. Chem., 56, 1132A, 1984.

36. Lianos, P.; Lang, J.; Strazielle, C.; Zana, R., Fluorescence Probe Study of Oil in Water Microemulsions. 1. Effect of Pentanol and Dodecane or Toluene on Some Properties of Sodium Dodecylsulfate Micelles, J. Phys. Chem., 86, 1019, 1986.

37. Mullins, F. G. P.; Kirkbright, G. F., Selectivity and Efficiency Measurements in High-Performance Liquid Chromatography using Hexadecyltrimethylammonium Bromide in the Mobile Phase, Analyst, 111, 1273, 1986.

38. Malliaris, A.; Lang, J.; Sturm, J.; Zana, R., Intermicellar Migration of Reactants: Effect of Additions of Alcohols, Oils, and Electrolytes, J. Phys. Chem., 91, 1475, 1987.

39. Soldi, V.; Magalhaes Erismann, N.; Quina, F. H., Micelle-Mimetic Ionene Polyelectrolytes, J. Amer. Chem. Soc., 110, in press, 1988.