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The Effect of Stationary-Phase Pore Size on Retention Behavior in Micellar Liquid Chromatography

Timothy J. McCormick,[†,‡] Joe P. Foley,[*,§] Christopher M. Riley,[†] and David K. Lloyd[†]

Department of Chemistry, Drexel University, Philadelphia, Pennsylvania 19104, Pharmaceutical Research and Development, The DuPont Pharmaceuticals Company, Wilmington, Delaware 19880, and Department of Chemistry, Villanova University, Villanova, Pennsylvania 19085

One of the limitations that has restricted the applicability of micellar liquid chromatography (MLC) is the weak eluting power of micellar mobile phases compared to conventional hydro-organic mobile phases used in reversed-phase liquid chromatography. This may be the result of Donnan or steric exclusion of the micelles from the pores of the stationary phase, within which nearly all (≥99%) of the stationary phase resides and the analytes spend most of their time. To determine whether wide-pore stationary phases would overcome this limitation in MLC, several C8 and C18 stationary phases ranging from 100 to 4000 Å were investigated using a diverse set of test solutes and micellar solutions of anionic, neutral, and cationic surfactants as mobile phases. With the larger pore size stationary phases, the eluting power of the MLC mobile phases was enhanced with all surfactant types, the greatest effect being with the neutral surfactant. Differences in retention behavior were observed between various solute types and between the C8 and C18 stationary phases. These differences appear to be related to the relative hydrophobicity of the solutes and to differences in the surfactant-modified stationary phases. Partitioning behavior of representative solutes on the large-pore C8 and C18 columns was shown to follow the three-phase partitioning model for MLC. Methylene group selectivity data showed only minor differences in the stationary-phase characteristics between the small- and large-pore size C18 columns. The true eluting power of micellar mobile phases was revealed with wide-pore stationary phases and was demonstrated by the separation and elution of an extended series of alkylphenones on C18 columns.

In 1980, Armstrong and Henry first reported on the use of an aqueous micellar solution as the mobile phase in high-performance liquid chromatography (HPLC) for the separation of phenols and polynuclear aromatic hydrocarbons.[1] Since that time, numerous other investigators have studied the unique properties and capabilities of micellar mobile phases in reversed-phase liquid chromatography (RPLC) including such aspects as retention behavior,[2−5] solvent strength and selectivity [4,6−8], efficiency,[9−16] gradient elution,[17−20] detection,[21−23] and direct injection of biological fluids.[24−29] Some of the main advantages of micellar liquid

* Corresponding author: (e-mail) jfoley@drexel.edu.
† The DuPont Pharmaceutical Co.
‡ Villanova University.
§ Drexel University.

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chromatography (MLC) are as follows: (i) simultaneous separation of charged and uncharged solutes, (ii) gradient elution with rapid equilibration, (iii) direct on-column injection of physiological fluids, (iv) unique separation selectivity, (v) enhanced luminescence detection, and (vi) low cost and safety.[30] In addition, MLC uses the same hardware (pumps, injectors, tubing, detectors, etc.) and columns as traditional RPLC, which makes it compatible with conventional HPLC systems.

Despite these advantages, MLC has not found widespread use due to poor column efficiency[30−36] and the apparently weak solvent strength of micellar eluents[4,11,12,30,32,34] compared to hydro-organic mobile phases. The problem of poor column efficiency has been studied extensively.[11−13,33,37,38] Reduced efficiency in MLC has been attributed to poor wetting of the hydrophobic stationary phase by the aqueous mobile phase,[12] slow mass transfer between the micelles and the stationary phase,[13] and poor mass transfer within the stationary phase itself.[33,37,38] The two main approaches that have been used to enhance efficiency in MLC are to add low amounts of 1-propanol to the mobile phase and to increase the column temperature.[31] Dorsey[11] found that the addition of 3% 1-propanol to the mobile phase and use of a column temperature of 40 °C gave column efficiencies equivalent to those of hydro-organic mobile phases and was still a sufficiently low concentration of organic solvent not to significantly perturb the micelle structure.

As for the weak solvent strength, Dorsey[32] suggested the use of shorter columns and shorter chain length stationary phases to overcome long analysis times in MLC. However, Foley[39] has shown in a critical compilation of solute−micelle binding constants that solute retention in MLC was excessive (k > 10) for more than one-fourth of the compounds examined when the surfactant concentration was adjusted to provide optimum selectivity. By design his study excluded most moderately to highly hydrophobic compounds; the fraction of excessively retained compounds would have been even larger if a truly representative set of compounds had been considered. Given this, the use of shorter columns and shorter chain length stationary phases may not be effective in overcoming the problem of weak solvent strength.

We believe that the apparent lack of strength of micellar mobile phases is due to the exclusion of the micelles from the pores, within which nearly all (≥99%) of the stationary phase resides and the analytes spend most of their time. Since the excluded micelles do not have direct access to the analytes except when the latter have diffused out of the pores, it is not surprising that even high concentrations of micelles are not sufficient to elute moderately to highly hydrophobic compounds. In the case of nonionic surfactants, steric effects are most likely the cause of micellar exclusion from small-pore materials, whereas with ionic surfactants, both electrostatic and steric effects are probably responsible for micellar exclusion. The monomer units of common surfactants such as sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) are known to adsorb quite heavily onto octadecyl-derivatized silica (ODS), and the resulting charge buildup on the stationary-phase surface within the pores gives rise to a Donnan-like potential that will tend to repel like-charged species from the pore, especially large structures such as micelles whose dimensions (typically 30−60 Å[22]) are commensurate with pore diameters of typical (small-pore) ODS phases most commonly used in RPLC.

In fact, Okada[40,41] used this principle as the basis for the technique he called “micelle exclusion chromatography” wherein micellar mobile phases (anionic and cationic) were utilized with a size exclusion column for the separation of cationic and anionic solutes, respectively. With a size exclusion column, the micelles are partly excluded by the stationary phase. The region where micelles can permeate is called the “external solution”. No micelles can permeate the inner part of the stationary phase; however, the surfactant monomers can permeate this region. Ion-pair or ion-interaction chromatographic retention is dominant in this region, called the “inner solution”.

In Okada's work, the micelles could permeate some portion of the stationary phase, while in the work of Herries et al.[42] and Terabe et al.[43] the micelles were completely excluded from the stationary phase. This total exclusion of the micelle despite a micellar weight (∼12 000) lower than the exclusion limit of the SEC column was reported to be due to Donnan exclusion or the electrostatic repulsion between the anionic micelle and SDS molecules adsorbed on the packing material.[43] Micelles are also excluded by molecular sieve effects according to their sizes.[44] In either case, the principle of micellar exclusion (partial or total) from the stationary phase was the basis for the separation mechanism. Therefore, it seems plausible that such a phenomenon would be expected to play a role in the separation mechanism of MLC and may, as we have asserted, be responsible for the reportedly weak solvent strength of micellar mobile phases. The purpose of the current work is to investigate whether micellar exclusion by the stationary phase is occurring in MLC and whether larger pore size columns can mitigate this effect.

EXPERIMENTAL SECTION

Apparatus. The chromatographic apparatus used was a Hewlett-Packard (Rockville, MD) HP 1090M Win liquid chromatography system equipped with a DR5 ternary gradient pump, a diode array detector, a temperature-controlled column compartment, and a variable-volume autoinjector. Studies using an SDS mobile phase were conducted on the following three sets of columns: YMC-Pack (YMC, Inc., Wilmington, NC) C18, 150 ×

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Table 1. Solute Concentrations and RPLC Mobile-Phase Compositions

solutes	concn (ppm)	% methanol YMC C18	% methanol EM C8	% methanol Alltech C18
I. alkylbenzenes				
toluene	108	60	40	40
ethylbenzene	108	60	50	50
propylbenzene	107	70	55	55
butylbenzene	107	70	60	60
II. alkylphenones				
acetophenone	128	35	25	20
propiophenone	63	45	35	30
butyrophenone	63	55	45	40
III. PAHs				
naphthalene	61	65	50	50
anthracene	25	75	60	60
IV. warfarin	110	60[a]	48[a]	45[a]

[a] The water portion of the mobile phase contained 2% acetic acid for ion suppression of warfarin.

4.6 mm, 5 μm, 120, 200, and 300 Å; LiChroCART (EM Separations, Gibbstown, NJ) LiChrospher C8, 250 × 4.0 mm, 10 μm, 100, 300, and 1000 Å; Alltech (Alltech Associates, Inc., Deerfield, IL) Macrosphere C18, 150 × 4.6 mm, 7 μm, 100, 300, 1000, and 4000 Å. Studies using polyoxyethylene 10-lauryl ether and dodecyltrimethylammonium bromide mobile phases were conducted on the Alltech Macrosphere C18 columns. Separation of a homologous series of alkylphenones was performed on Alltech Nucleosil C18 columns, 150 × 4.6 mm, 7 μm, 100 and 1000 Å.

Reagents, Chemicals, and Solutions. SDS 99%, polyoxyethylene 10-lauryl ether (Brij-22), and dodecyltrimethylammonium bromide 99% (DTAB) were obtained from Sigma Chemical Co. (St. Louis, MO), HPLC grade methanol was obtained from EM Science (Gibbstown, NJ), HPLC grade water was obtained from a Milli-Q Plus water system (Millipore Corp., Milford, MA). The test solutes were obtained from various sources. The test solutes were prepared as dilute solutions in methanol. The concentrations of the various solutes are given in Table 1 along with the RPLC mobile-phase composition used for each solute.

Procedure. Ten test solutes of varying polarity and size (e.g., alkylbenzenes, alkylphenones, PAHs, and warfarin) were studied on three sets of columns (YMC C18, EM C8, and Alltech Macrosphere C18) using an SDS mobile phase. All of the test solutes had an UV chromophore for ease of detection. In addition, limited studies were carried out on the Alltech Macrosphere C18 columns using Brij-22 and DTAB mobile phases, with one nonpolar solute pair (propylbenzene and butylbenzene), one polar pair (propiophenone and butyrophenone), and warfarin as test solutes. Separation of a test mixture of a homologous series of alkylphenones was performed on Alltech Nucleosil C18 100- and 1000-Å columns using RPLC and MLC gradient conditions to demonstrate the advantage of the wide-pore stationary phase.

A solution of sodium nitrate in water (~0.1 mg/mL) was used as the void time (t_o) marker in both the RPLC and MLC experiments except with the cationic surfactant (DTAB) mobile phase where water was used as the t_o marker. Acetone was initially chosen as the void time (t_o) marker; however, after further investigation it was apparent that acetone was being retained and therefore was unsuitable as a t_o marker. Sodium nitrate and water were subsequently selected as the void time probes since they performed well as unretained t_o markers. The RPLC mobile phase used for t_o determinations was 50/50 methanol/water, and the MLC t_o mobile phases used were as follows: 300 mM SDS, 50 mM Brij-22, and 100 mM DTAB. Sodium perchlorate (100 mM) was added to the RPLC and MLC mobile phases that were used for the t_o determinations with sodium nitrate in order to shield the negative silica surface charge. This was done to prevent Donnan exclusion of the negative nitrate ion from the stationary-phase pores, which would lead to erroneously low t_o values.[45]

The solutes were injected in triplicate for the RPLC experiments and in duplicate for the MLC experiments. The RPLC mobile phases consisted of a mixture of methanol and water used in various proportions for each of the test solutes (Table 1). For each set of columns, RPLC scouting experiments were performed using the smallest and largest pore size columns in the set in order to determine a mobile-phase composition that would yield a k_{RPLC} between approximately 1 and 20 across that set of columns. In this way, the RPLC mobile-phase composition was determined for each solute on each set of columns so that a constant mobile-phase composition could be used for each solute across a particular set of columns. The RPLC mobile-phase composition varied between solutes for a particular set of columns, but not between columns for a particular solute. A similar scouting process was used to determine the surfactant concentration for the MLC mobile phase for each set of columns. However, it was not necessary to vary the MLC mobile-phase composition between solutes in order to keep k_{MLC} between approximately 1 and 20 for a given set of columns. The same surfactant concentration was used for each solute on a given set of columns. Using the above process, the MLC mobile phases selected were 300 mM SDS (cmc = 8.2 mM) for the YMC columns and 100 mM SDS for the EM and Alltech columns. For the limited studies on the Alltech columns, 50 mM Brij-22 (cmc = 0.09 mM) and 100 mM DTAB (cmc = 16 mM) were selected as mobile phases. All data were acquired at a flow rate of 1.5 mL/min (except for the RPLC and MLC gradients below, which were run at a flow rate of 1.0 mL/min), column temperature of 40 °C, injection volume of 20 μL, and detector settings of 210 and 254 nm (for SDS and Brij-22 mobile phases). Detector settings of 235 and 254 nm were used for DTAB mobile phase.

Eleven alkylphenones (acetophenone to dodecanophenone) were combined and dissolved in methanol to form a test mixture containing 0.01% v/v or w/w of each alkylphenone. The test mixture was run under both RPLC and MLC gradient conditions on the Alltech Nucleosil C18 100- and 1000-Å columns. The RPLC gradient was a linear % B gradient from 10 to 100% methanol in 30 min. The MLC gradient was a linear pM gradient from 12.5 to 258.2 mM SDS (containing 5% 1-propanol) in 30 min, where pM = −log [M] and [M] = ([surfactant] − cmc)/N with N being the surfactant aggregation number. In this type of gradient, the concentration of micelles (M) in the mobile phase increases exponentially with time instead of linearly as in a % B gradient. In this case, ΔpM was −0.3 and ΔpM/Δt was −0.06 where t is time in minutes. Linear pM gradients are preferred in secondary chemical equilibrium (SCE) systems, of which MLC is one, because they give a roughly linear decrease in log k which is

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analogous to a linear solvent strength gradient in RPLC.

RESULTS AND DISCUSSION

Treatment of Retention Data. As the pore size of a porous material of a given particle diameter is increased, the specific surface area is decreased. As a consequence, the volume of the bonded stationary phase and thus the stationary phase-to-mobile phase ratio is also decreased. Therefore, under equal mobile-phase conditions the retention (k) of a solute on a large-pore column will be smaller than on an otherwise identical small-pore column since $k = K\phi$ where K is the partition coefficient and ϕ is the phase ratio. Partition coefficients are affected by such variables as the nature of the solute, mobile-phase composition, stationary-phase type, and temperature.[46] Since all of these variables were held constant for each particular solute and set of columns, the partition coefficients of the solutes were taken to be invariant between columns. Thus, variation in k with pore size can be factored out by referencing the retention of a solute measured under MLC conditions on a given column to the retention of that solute obtained under conventional RPLC conditions on the same column. That is, k_{MLC}/k_{RPLC} can be evaluated for each solute on each set of columns.

For a given pore size and stationary phase, the above ratio of retention factors, k_{MLC}/k_{RPLC}, could be greater or less than 1, depending on the mobile-phase composition (eluting strength) chosen for the MLC and RPLC experiments; it may also vary slightly from test solute to test solute due to solute-specific differences in the eluting strength of the MLC and RPLC mobile phases. Similarly, for a given test solute and a set of MLC and RPLC mobile-phase conditions, the retention ratio could differ somewhat from one type or brand of stationary phase to another.

To facilitate a comparison of k_{MLC}/k_{RPLC} obtained for several test solutes using different sets of columns and mobile-phase conditions, it is convenient to normalize in some way the MLC and RPLC retention factors before calculating their quotient. For a given type and brand of column with different pore sizes, the retention factor of each solute was divided by the retention factor of the same solute observed on the column with the smallest pore size (in that set of columns). Thus, $k^*_{RPLC} = k_{RPLC,\ any\ pore\ size}/k_{RPLC,\ smallest\ pore\ size}$ and $k^*_{MLC} = k_{MLC,\ any\ pore\ size}/k_{MLC,\ smallest\ pore\ size}$, where the asterisk indicates a normalized retention factor. Using the above definition, the normalized retention factor of any solute is unity on the smallest pore size column of a given set.

By then graphing the ratio of *normalized* MLC retention to *normalized* RPLC retention (k^*_{MLC}/k^*_{RPLC}) for the solute(s) of interest *as a function of pore size* for a given set of columns, it is easy to discern whether the reduction in k_{MLC} with increasing pore size is greater than what would be predicted based only on decreasing stationary-phase volume (phase ratio) as the pore size increases. Values of unity for k^*_{MLC}/k^*_{RPLC} would indicate that the observed reduction in k^*_{MLC} with increasing pore size was due only to the reduction of the stationary-phase volume (phase ratio), whereas values less than unity (a greater than expected reduction in k^*_{MLC}) would indicate that the larger pore size phases are allowing better penetration of the micelles into the pores such that they can reach the solute at the internal surface of the stationary phase better and thus elute the solutes in less time.

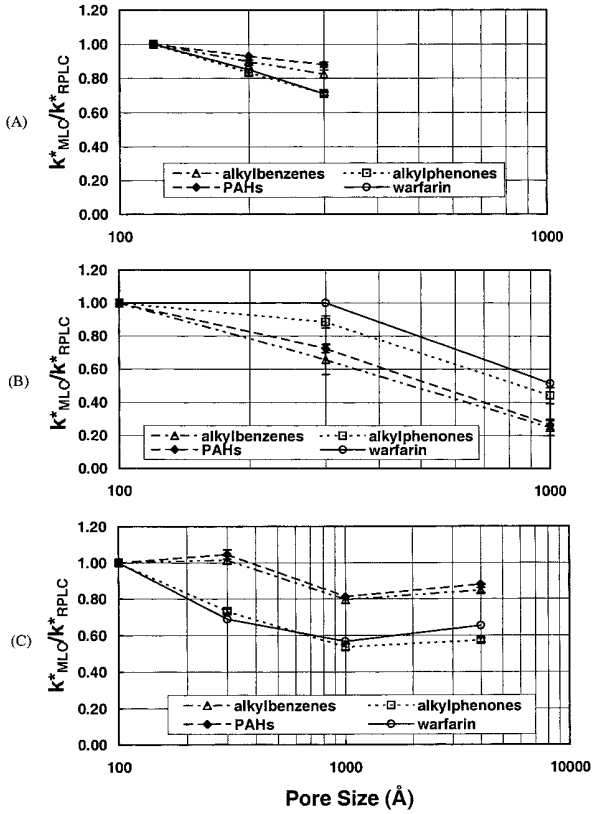

Figure 1. Normalized retention data versus pore size using SDS mobile phase with (A) YMC C18, (B) EM C8, and (C) Alltech C18 columns.

Due to the adsorption of surfactants onto the stationary phase of RPLC columns, all reference RPLC experiments for a given column were completed prior to the use of micellar mobile phases. Also, after each surfactant study, the columns were rinsed for ≥3 h with methanol to remove the adsorbed surfactant from the stationary phase before moving on to the next surfactant.

Studies with SDS Mobile Phase. *YMC, EM, and Alltech Columns.* The results of the above analyses for each set of columns using SDS mobile phase are plotted in Figure 1. The data show that the relative drop in k_{MLC} exceeds the relative drop in k_{RPLC} for the higher pore size columns in each set of columns. The k^*_{MLC}/k^*_{RPLC} values for the larger pore size columns are less than unity and the reduction in k^*_{MLC} is greater than what would be expected on the basis of a reduction of the phase ratio only. This indicates that the larger pore size phases are allowing better penetration of the micelles into the pores such that they can reach the solute at the internal surface of the stationary phase and thus elute the solutes in less time.

The data also show certain trends in the solute behavior. Generally, the nonpolar solutes (alkylbenzenes and PAHs) behave similarly as a distinct group and the more polar solutes (alkylphenones and warfarin) behave similarly as another distinct group. Also, on the C18 columns (YMC and Alltech), the more hydrophobic compounds (alkylbenzenes and PAHs) tend to give higher k^*_{MLC}/k^*_{RPLC} values than the less hydrophobic compounds (alkylphenones and warfarin) whereas on the C8 columns (EM) the

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Table 2. Calculation of MLC Partition Coefficients: (from $1/k$ vs [M] plots using SDS)

pore size (Å)	phase ratio	intercept	std error	P_{SW}	slope	std error	P_{MW}	P_{SM}
			EM C8 Columns. I. Ethylbenzene					
100	9.7×10^{-2}	1.72×10^{-3}	1.01×10^{-3}	6.0×10^{3}	3.78×10^{-1}	5.57×10^{-3}	8.9×10^{2}	6.7
300	1.4×10^{-2}	4.63×10^{-3}	4.87×10^{-3}	*a*	2.80	2.68×10^{-2}	*a*	*a*
1000	6.0×10^{-3}	1.39×10^{-1}	1.20×10^{-2}	1.2×10^{3}	1.85×10^{1}	2.33×10^{-1}	5.4×10^{2}	2.2
			II. Propiophenone					
100	9.7×10^{-2}	1.23×10^{-2}	1.41×10^{-3}	8.4×10^{2}	4.68×10^{-1}	7.77×10^{-3}	1.6×10^{2}	5.4
300	1.4×10^{-2}	9.33×10^{-2}	1.54×10^{-2}	7.7×10^{2}	2.92	8.48×10^{-2}	1.3×10^{2}	6.0
1000	6.0×10^{-3}	1.64	2.90×10^{-2}	1.0×10^{2}	8.19×10^{1}	2.65	2.0×10^{2}	5.0×10^{-1}
			Alltech C18 Columns. I. Ethylbenzene					
100	1.6×10^{-1}	1.70×10^{-3}	4.15×10^{-4}	3.7×10^{3}	3.94×10^{-1}	2.28×10^{-3}	9.4×10^{2}	3.9
300	1.0×10^{-1}	2.30×10^{-4}	1.19×10^{-3}	*a*	5.85×10^{-1}	6.54×10^{-3}	*a*	*a*
1000	2.2×10^{-2}	1.83×10^{-2}	1.31×10^{-3}	2.5×10^{3}	3.26	2.54×10^{-2}	7.2×10^{2}	3.4
4000	1.0×10^{-2}	2.65×10^{-2}	2.30×10^{-3}	3.8×10^{3}	4.98	4.49×10^{-2}	7.7×10^{2}	4.9
			II. Propiophenone					
100	1.6×10^{-1}	9.58×10^{-3}	9.43×10^{-4}	6.6×10^{2}	3.29×10^{-1}	5.19×10^{-3}	1.4×10^{2}	4.7
300	1.0×10^{-1}	1.94×10^{-2}	2.46×10^{-3}	5.1×10^{2}	7.38×10^{-1}	1.35×10^{-2}	1.6×10^{2}	3.2
1000	2.2×10^{-2}	8.59×10^{-2}	3.54×10^{-3}	5.3×10^{2}	6.34	6.90×10^{-2}	3.0×10^{2}	1.8
4000	1.0×10^{-2}	1.23×10^{-1}	6.57×10^{-3}	8.2×10^{2}	8.78	1.28×10^{-1}	2.9×10^{2}	2.8

[a] Not calculated since the standard error in the intercept is greater than the intercept.

reverse is true. The more hydrophobic compounds may give higher k^*_{MLC}/k^*_{RPLC} values on the C18 columns since there is probably not much partitioning of these compounds into the bulk aqueous phase. As discussed below, three-way partitioning of the solute between the aqueous, micellar, and stationary phases controls retention behavior in MLC. While retention of more polar compounds is determined by their partitioning from the bulk aqueous phase into the micelle and stationary phases, the more hydrophobic compounds might be directly transferred from the micellar phase into the stationary phase.[30]

Determination of Partitioning Behavior on EM and Alltech Columns. Armstrong and Nome described the partitioning behavior of solutes eluted with micellar mobile phases in liquid chromatography.[47] A three-phase or three-way partitioning model was presented to account for the partitioning of the solute between the micelle and water, between the stationary phase and water, and between the stationary phase and micelle. Arunyanart and Cline Love proposed a three-phase equilibrium model to describe solute retention in MLC based on the equilibria of the solute between the bulk phase−micelle, bulk phase−stationary phase, and micelle−stationary phase.[48] The Armstrong and Nome and Arunyanart and Cline Love equations can be rewritten as follows:[49]

$$\frac{1}{k} = \frac{K_{AM}}{\phi P_{SW}}[M] + \frac{1}{\phi P_{SW}} \quad (1)$$

where k is the retention factor, [M] is the total concentration of surfactant in the mobile phase minus the cmc, ϕ is the phase ratio of the column, K_{AM} is the solute−micelle binding constant, and P_{SW} is the partition coefficient between the stationary phase and water. Plots of $1/k$ versus [M] should yield a straight line. The values of P_{SW} and K_{AM} are obtained from the intercept and slope of the plot, respectively. The value of P_{MW}, the partition coefficient of a solute between the micelle and water, is obtained from the following relationship:[50]

$$K_{AM} = V(P_{MW} - 1) \quad (2)$$

where V is the molar volume. The quantity P_{SM}, the partition coefficient of a solute between the stationary phase and the micelle, is obtained from the ratio of the other two partition coefficients [50]

$$P_{SM} = P_{SW}/P_{MW} \quad (3)$$

To further investigate the trend reversal in k^*_{MLC}/k^*_{RPLC} values observed between the C18 and C8 columns, retention on the EM C8 and Alltech C18 columns was evaluated with the SDS mobile phase in terms of the three-way partitioning model, according to eq 1 above. Representative nonpolar (ethylbenzene) and polar (propiophenone) solutes were utilized in the evaluation. If the three-phase model properly describes the behavior of solutes, then plots of $1/k$ versus [M] should be linear. Indeed, linear plots (correlation coefficients ≥ 0.998, $n = 4$) of $1/k$ versus [M] were obtained for each solute on both sets of columns.

The various MLC partition coefficients (Table 2) were also calculated from the EM C8 and Alltech C18 columns' data using eqs 1−3 above. To determine the phase ratio, the stationary-phase volume was calculated according to the method of Sentell and Dorsey[51] and the mobile-phase volume was calculated from the product of the void time and flow rate. The P_{SW} and P_{MW} values for ethylbenzene are greater than the corresponding values for propiophenone on both the C8 and C18 columns, which is consistent with their relative hydrophobicity. For ethylbenzene chromatographed on the 300-Å column, the partition coefficients

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Table 3. Ratio of Phase Ratios (ϕ_{1000}/ϕ_{100})

		RPLC		MLC	
column	calcd	ethyl-benzene	propio-phenone	ethyl-benzene	propio-phenone
C8	0.06	0.07	0.06	0.012	0.0075
C18	0.14	0.13	0.11	0.09	0.11

could not be calculated because of the large error in the intercept. It can also be seen from Table 2 that, with the C8 column, there is a large decrease in P_{SW} on going from the 100- to 1000-Å pore sizes. This suggests that the C8 stationary phase is modified differently by the SDS surfactant monomers in the different pore size columns. This hypothesis is supported if one looks at the phase ratios, which can be calculated for each column.

The intercepts in Table 2 are equal to the reciprocal of the retention factor that would be measured if the micelle concentration were zero as determined by extrapolation from all the experimental data. Since k is directly proportional to the phase ratio, then the ratio of k calculated as 1/intercept for two different columns in Table 2 would be equal to the ratio of the phase ratios of those columns. Table 3 shows the ratio of phase ratios, ϕ_{1000}/ϕ_{100}, where the subscripts 1000 and 100 refer to the pore sizes of the columns. The calculated ϕ_{1000}/ϕ_{100} were determined as described above.[51] ϕ_{1000}/ϕ_{100} was also determined from the RPLC k data for ethylbenzene and propiophenone, as the ratio of the retention factors determined on the different pore size columns. The fifth and sixth columns in Table 3 show the ratio ϕ_{1000}/ϕ_{100} as determined from the intercepts of the $1/k$ vs [M] plots, for both ethylbenzene and propiophenone. It can be seen that the calculated ratio ϕ_{1000}/ϕ_{100} and the experimental values from RPLC are in good agreement for both the C8 and C18 data. The ratio ϕ_{1000}/ϕ_{100} determined in the MLC experiments with the C18 columns is also in reasonable agreement with the other values. However, the ratio determined on the C8 column is very different, as measured using both ethylbenzene and propiophenone. This suggests that relatively more SDS surfactant monomer is adsorbed in the 100-Å C8 column (thus effectively increasing ϕ_{100}) or relatively little is adsorbed on the surface in the 1000-Å column (thus effectively decreasing ϕ_{1000}). Thus, the reversal in elution strength of the micellar eluents between the EM C8 and Alltech C18 columns (Figure 1) may be explained by differences in the adsorption of the surfactant monomer onto the stationary phase.

Studies with Brij-22 and DTAB Mobile Phases. Alltech Columns. On the basis of the results from the three sets of columns using SDS mobile phase, the Alltech columns were selected for further studies with Brij-22 and DTAB mobile phases because of their larger pore size range. A representative nonpolar solute pair (propyl- and butylbenzene), a polar solute pair (propio- and butyrophenone), and warfarin were used to study the Brij-22 and DTAB mobile phases with the Alltech columns. Solute pairs were utilized in order to obtain data on methylene group selectivity.

The results of these limited studies are plotted in Figure 2. The data show enhanced MLC performance with the larger pore size stationary phases for both Brij-22 and DTAB surfactants, which is consistent with the SDS surfactant data. The largest effect is seen with the nonionic surfactant Brij-22. With the ionic

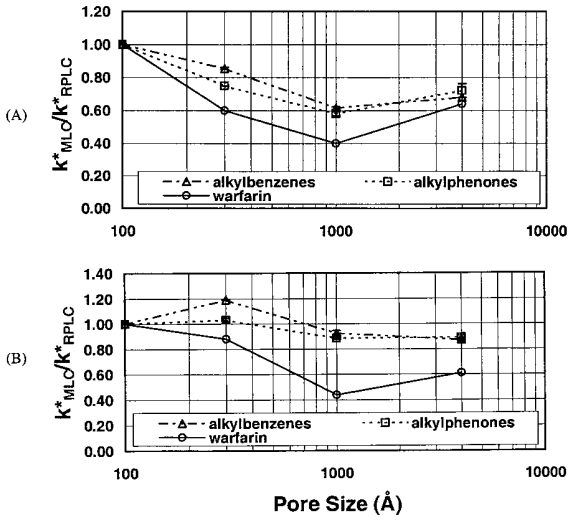

Figure 2. Normalized retention data versus pore size using Alltech columns with (A) Brij-22, and (B) DTAB mobile phase.

surfactants (SDS and DTAB) there appears to be some Donnan exclusion of the micelle from the smaller pore sizes (≤300 Å) as evidenced by some k^*_{MLC}/k^*_{RPLC} values that are greater than unity. This is most likely due to the adsorption of the ionic surfactant monomers on the surface of the stationary phase and the resulting electrostatic repulsion of the ionic micelles from the pores. This exclusion does not appear to occur with the nonionic Brij-22 surfactant, even though it too adsorbs to the stationary phase, since there are no electrostatic effects with this surfactant. In addition, the Brij-22 and DTAB data show trends in solute behavior similar to that of the SDS data. That is, the more hydrophobic compounds typically give higher k^*_{MLC}/k^*_{RPLC} values, probably for the same reason discussed for the SDS data.

Methylene Group Selectivity. Methylene group selectivity, α_{CH_2}, is defined as

$$\alpha_{CH_2} = k_{nc}/k_{nc-1} \quad (4)$$

where nc and nc − 1 indicate k values for two homologues differing by a single carbon unit.[52] Methylene group selectivity is independent of the phase ratio of the column.[53] Methylene group selectivity is only affected by variables that affect the partition coefficients[46] (i.e., solute type, mobile-phase composition, stationary-phase type, and temperature). For a given solute pair that differ only by one methylene unit, α_{CH_2} should remain essentially constant across a set of columns if only the phase ratio is changing since the mobile-phase composition and temperature were kept constant. Any differences observed for α_{CH_2} will be due to differences in the stationary phase.[53]

Methylene group selectivity data for the Alltech columns under RPLC and MLC conditions are shown in Table 4 for the nonpolar solute pair butylbenzene/propylbenzene and the polar solute pair butyrophenone/propiophenone. The α_{CH_2} data were calculated based on eq 4. The data for the nonpolar solute pair (butyl-

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Table 4. Methylene Group Selectivity: Alltech C18 Columns

solute pair	pore size (Å) 100	300	1000	4000
1. RPLC				
butylbenzene/propylbenzene	1.90	1.92	1.90	1.90
butyrophenone/propiophenone	2.23	2.37	2.39	2.38
2. MLC/SDS				
butylbenzene/propylbenzene	1.21	1.24	1.23	1.17
butyrophenone/propiophenone	1.32	1.42	1.44	1.42
3. MLC/Brij-22				
butylbenzene/propylbenzene	1.08	1.09	1.09	1.08
butyrophenone/propiophenone	1.36	1.44	1.43	1.40
4. MLC/DTAB				
butylbenzene/propylbenzene	1.20	1.22	1.24	1.23
butyrophenone/propiophenone	1.36	1.46	1.49	1.48

Table 5. Surface Coverage: Alltech C18 Columns

pore size (Å)	P_c	S (m^2/g)	N^a ($\mu mol/m^2$)
100	14	350	2.2
300	10	100	5.2
1000	2.5	25	4.8
4000	1	10	4.7

[a] Calculated via eq 5. M = 253 and n_c = 18 for a C18 bonded moiety.

benzene/propylbenzene) are essentially constant across the four columns under RPLC and MLC conditions, which indicates that the stationary phase appears consistent for these solutes except for the phase ratio. However, for the polar solute pair (butyrophenone/propiophenone), α_{CH_2} shows an increase of about 6−8% in going from the 100-Å to the 300-Å column after which it is essentially constant up to the 4000-Å column. This may be due to minor differences in the stationary-phase surface characteristics between the small-pore and wide-pore columns.

In general, column selectivity is directly related to bonded phase surface coverage.[54] For monomeric phases, the bonding density or surface coverage can be calculated as follows:[54]

$$N = \frac{10^6 P_c}{1200 n_c - P_c(M-1)} \frac{1}{S} \qquad (5)$$

where N is the surface coverage (in $\mu mol/m^2$), P_c is the percent carbon of the bonded phase, n_c is the number of carbons in the bonded silane molecule, M is the molecular weight of the bonded silane molecule, and S is the specific surface area of the unbonded silica (in m^2/g). The surface coverage was calculated for each of the Alltech columns, and the data (Table 5) show that the 100-Å column had less surface coverage than the other columns. Also, the 300-, 1000-, and 4000-Å columns all had approximately the same surface coverage. This would account for the trend in α_{CH_2} seen with the polar solutes on the Alltech columns. The methylene or hydrophobic selectivity value represents the free energy of transfer of a methylene group from the mobile phase to the nonpolar stationary phase.[4] The hydrophobic selectivity decreases as the difference between mobile- and stationary-phase "polarities" is reduced. Since the 100-Å column has less surface coverage than the other columns, the stationary-phase surface of this column may be more polar than the surface of the other columns and therefore a methylene group of the alkylphenones will undergo less of a change in its microenvironment "polarity" on the 100-Åcolumn than it will on the other columns. The difference in stationary-phase surface polarities between the small-pore versus large-pore columns is most likely due to a greater concentration of surface silanols on the small-pore column since the surface coverage of the bonded phase is less for this column.

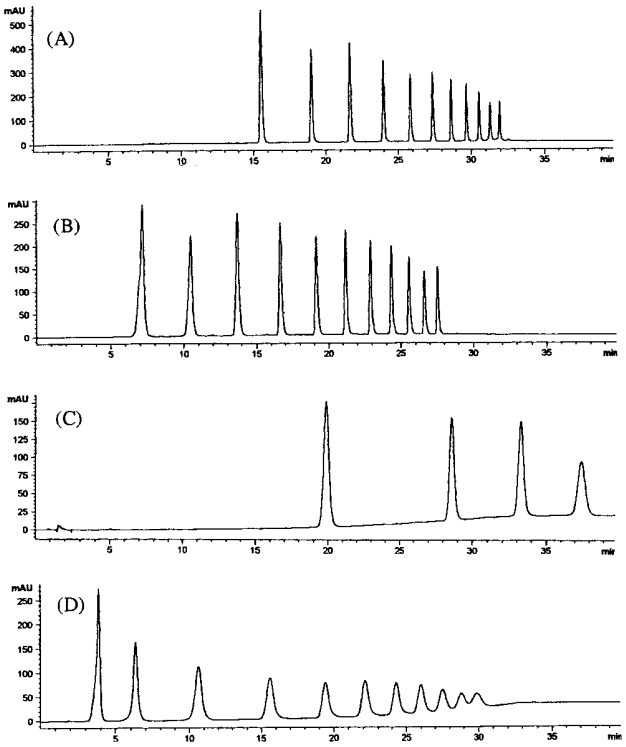

Figure 3. Separation of a homologous series of alkylphenones under (A) RPLC conditions on Alltech 100-Å column, (B) RPLC conditions on Alltech 1000-Å column, (C) MLC conditions on Alltech 100-Å column, and (D) MLC conditions on Alltech 1000-Å column. The 254-nm signals are shown.

Separation of a Series of Alkylphenones. Figure 3 shows the separation of a homologous series of alkylphenones on the Alltech 100 and 1000-Å columns under RPLC and MLC gradient conditions. Under RPLC conditions, all 11 alkylphenones are separated within the 30-min gradient window on the 1000-Å column whereas only the first 8 in the series are eluted within the gradient window on the 100-Å column with the remaining 3 eluting only after 100% methanol is reached. This is due to the drop in the stationary-phase volume as the pore size is increased. As expected, the first peak (acetophenone) shows the most change in retention time, about a 54% decrease, going from the 100- to 1000-Å column.

Under MLC conditions, all 11 alkylphenones are eluted on the 1000-Å column whereas only the first 4 are eluted on the 100-Å column. This is due to the enhanced eluting power of the MLC mobile phase on the larger pore size stationary phase. The retention time for acetophenone decreased ~80% going from the 100- to 1000-Å column, which exceeds what would be expected based on the decrease in the stationary-phase volume alone. Again,

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this demonstrates the potential advantage of using the wide-pore stationary phases in overcoming the weak eluting power limitation in MLC. In addition, this is apparently an improvement over previously reported results since the highest homologues that have been reported in MLC studies using alkylphenones were valerophenone and hexanophenone.[4,5,39]

CONCLUSION

The results indicate that the micelles are penetrating the pores better on the wider pore stationary phases and are eluting the solutes faster, taking into account the unavoidable drop in retention factor with increasing pore size, than what can be achieved on the smaller pore stationary phases. Thus, it appears that wide-pore stationary phases may be useful in overcoming one of the important limitations in MLC.

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