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Stationary-phase effects on efficiency in micellar liquid chromatography

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Abstract

One of the main limitations of micellar liquid chromatography (MLC) is the lower efficiency compared to reversed-phase liquid chromatography (RPLC) with hydro-organic mobile phases. The main contribution to the reduced efficiency has been shown to be due to the slow mass transfer between micelles, the aqueous phase, and the stationary phase mainly due to surfactant adsorption onto the stationary phase. The use of a variety of stationary phases, including large-pore short alkyl chain, non-porous, superficially porous, and perfluorinated, is shown to have differing effects on remediation of the reduced efficiency. Diffusion coefficients were determined by the Taylor–Aris dispersion technique for the construction of Knox plots. The Knox plots are used to compare the efficiency data obtained with the different columns using several alkylphenones in both micellar mobile phase and hydro-organic mobile phase.

Keywords: Micellar liquid chromatography; Efficiency; Diffusion coefficients; Stationary phases, LC; Alkylphenones

1. Introduction

Micellar liquid chromatography (MLC) has been plagued by two main problems compared to reversed-phase liquid chromatography (RPLC) with hydro-organic mobile phases: (i) the excessive retention observed for hydrophobic compounds due to the weak eluting power of micellar mobile phases when used with conventional porous HPLC stationary phases [1–6]; and (ii) reduced efficiency due to one or more causes of slower mass transfer and/or flow anisotropy [2–4,7–13]. With respect to excessive retention of hydrophobic compounds, we recently reported the advantages of using stationary phases with large pore diameter, or "wide-pore stationary phases" in overcoming the perceived weak eluting power associated with micellar mobile phases [14]. We also showed that this wide-pore stationary-phase approach to eluting hydrophobic compounds in MLC is compatible with one of MLC's most important advantages: the direct sample introduction of biological fluids [15]. However, the problem of reduced efficiency in MLC still remains [3,4] despite extensive study [2–4,7–13]. Reduced efficiency in MLC has been attributed to several factors, including (i) poor wetting of the hydrophobic stationary phase by the aqueous mobile phase [2]; (ii) slow mass transfer between the micelles, the bulk aqueous phase, and the stationary phase; and (iii) dynamic modification of the stationary phase due to surfactant adsorption [3,4,8,16–23], which further reduces mass transfer within the stationary phase.

The contributions to the total peak variance in MLC can be described as follows:

$$\sigma^2_{\text{tot}} = \sigma^2_{\text{inj}} + \sigma^2_{\text{det}} + \sigma^2_{\text{eddy}} + \sigma^2_{\text{diff,mp}} + \sigma^2_{\text{diff,sp}} + \sigma^2_{\text{mt,sp}} + \sigma^2_{\text{mt(interstitial)}} + \sigma^2_{\text{mt(intraparticle)}} \quad (1)$$

where the total peak variance (σ^2_{tot}) is the sum of the variances due respectively to sample injection, detection, eddy dispersion (or flow anisotropy), diffusion of the solute in the mobile phase, diffusion of the solute in the stationary phase, stationary-phase mass transfer, mobile-phase mass transfer (between pores or interstitial), and stagnant mobile-phase mass transfer (within the pores, or intraparticle).

Based on our previous work with wide-pore stationary phases, we showed that retention could be greatly

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reduced through the use of wide-pore stationary phases due to the improved access of the micelles to the pores. Since nearly all (≥99%) of the stationary-phase surface area is within the pores, the analytes spend most of their time within the pores. With conventional porous HPLC phases (pore diameters of 150 Å or less), the micelles are largely excluded from the pores by steric constraints and therefore do not have access to the analytes except when they have diffused out of the pores into the interstitial region. In addition to steric constraints, it is well known that surfactant monomers adsorb onto the stationary phase [5,7,8,12,19,21,22]. When ionic surfactants are employed, the resulting charge buildup on the stationary phase 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 [24]) are commensurate with pore diameters of typical (small-pore) ODS phases most commonly used in RPLC. In the case of non-ionic 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 wide-pore solution to the excessive retention of hydrophobic compounds in MLC may or may not exacerbate the problem of poor efficiency for these compounds. One perspective is focused on micelle penetration into the pores (or not) and the subsequent effect on solute diffusion. With conventional pore size bonded phase silica, in which the micelles are excluded from the pores [14], intraparticle mass transfer of all solutes presumably occurs via diffusion of those solutes in their free states, which will be relatively rapid since diffusion coefficients of small-to-moderately sized unbound molecules are relatively large. In contrast, when large-pore phases are employed, the micelles are able to penetrate the pores and interact with the solutes, reducing their effective diffusion coefficient and thus slowing the solutes' intraparticle mass transfer.

Another perspective is centered around a possible kinetic barrier to the mass transfer of hydrophobic compounds due to a thermodynamically unfavorable "intermediate" state: some studies have suggested that hydrophobic compounds do not participate significantly in the aqueous component of the three-way partitioning scheme [7]. That is, they spend most of their time in the stationary phase or bound to the micelle, and very little time in the free state in the bulk aqueous phase because they are hydrophobic. If this is correct, then what happens when the pore size is such that the micelles are excluded by steric and/or Donnan exclusion effects? Is the intraparticle mass transfer of a hydrophobic solute slower than might otherwise be expected since the compound could only diffuse within the pore in its (thermodynamically unfavorable) free state? If so, then when wide-pore phases are employed, is the effect of the lower effective solute diffusion coefficient described in the first perspective counterbalanced for hydrophobic compounds by the removal of the requirement that they must diffuse in a thermodynamically unfavorable free state?

The two main approaches that have been used to enhance efficiency in MLC are to add small amounts of alcohol to the micellar mobile phase and to increase the column temperature [2,5,10,17,25,26]. Dorsey et al. [2] found that the addition of 3% 1-propanol to the mobile phase and use of a column temperature of 40 °C gave column efficiencies approaching those of hydro-organic mobile phases. Bailey and Cassidy have stated that the low efficiencies are not due to the mass transfer effects related to adsorbed surfactant on the stationary phase, but instead, the efficiency improvements from alcohol addition are related to effects within the mobile phase [11]. However, several other studies have explored the role of surfactant adsorption on reduced efficiency [3,4,8,16–23]. Others have studied the effects of varying the concentrations and types of alcohols to attempt to reduce the amount of surfactant adsorbed onto the stationary phase [2,8,10,17,25].

For the purposes of our experiments, 5% 1-propanol was added to the mobile phases and the columns were thermostated to 40 °C, as these conditions are generally recognized as being among the best to promote efficiency in MLC. The use of elevated temperatures was shown by Lavine [26] to increase efficiency in MLC purportedly due both to (i) a shift in the equilibrium of the solute away from the micelle and toward the bulk solvent, and to (ii) a decrease in the adsorbed surfactant on the stationary phase. The effects of the addition of alcohols into micellar mobile phases should also be mentioned. Zana and co-workers have extensively studied the effects of alcohols on various properties of micelles [27–32]. In general, the addition of short-chain alcohols (methanol–propanol) decrease the size, critical micelle concentration (CMC), and aggregation number (N) of ionic surfactants. A 7% (v/v) addition of 1-propanol was reported to reduce the CMC of sodium dodecyl sulfate (SDS) from 8.2 to 3.8 mM [32,33]. The primary purpose of the addition of alcohol is to improve on the poor wetting of the stationary phase, as well as to reduce the amount of adsorbed surfactant on the stationary phase. However, the addition of alcohols may also shift the equilibrium of the solute away from the micelle and toward the bulk solvent. These factors should be considered when interpreting the results and when making comparison to micellar mobile phases without increased temperature or the addition of alcohols. The use of these well-established conditions was intentional in order to determine whether further improvements in efficiency could be made based on the selection of stationary phase packing and silica pore size.

The use of the large-pore columns allows for reduced retention of hydrophobic compounds, so that higher alkylphenone homologues (heptanophenone, octanophenone, and nonaphenone) were eluted with reasonable retention factors (k) for all columns evaluated except the Zorbax OSD 70 Å, which was employed in conventional pore-size control experiments where large retention factors in MLC were expected.

2. Experimental

2.1. Equipment

An Agilent (Rockville, MD, USA) HP1100 Liquid Chromatograph system equipped with an in-line mobile-phase degasser, quaternary gradient pump, diode array detector, column thermostat, and a variable volume autosampler was used for all experiments. Control of the chromatograph and integration were performed using Agilent Chemstation software, version A.06.04. Studies were conducted using hydro-organic mobile phases as well as micellar mobile phases over an array of seven HPLC columns. The HPLC columns used were: Zorbax ODS (Agilent Technologies, Wilmington, DE, USA) 250 mm × 4.6 mm, 5 μm, 70 Å; Nucleosil C_4 (Phenomenex, Torrance, CA, USA) 250 mm × 4.6 mm, 7 μm, 1000 Å; Kovasil MS-C_{14} (CU Chemie Uetikon, Uetikon, Switzerland) 33 mm × 4.6 mm, 1.5 μm, non-porous; Zorbax Poroshell 300SB C_{18}, C_8, and C_3 (Agilent Technologies, Wilmington, DE, USA), 75 × 2.1 mm, 5 μm, 300 Å; and FluoroSep-RP Octyl (ES Industries, West Berlin, NJ, USA) 100 mm × 4.6 mm, 5 μm, 1000 Å.

2.2. Reagents, chemicals, and solutions

SDS ultrapure bioreagent (100%) was obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA), HPLC-grade 1-propanol and methanol were obtained from Allied Signal, Burdick and Jackson (Muskegon, MI, USA), HPLC grade water was obtained from a Milli-Q Plus water system (Millipore, Milford, MA), USP grade nitromethane used as a t_0 marker was obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA), test solutes acetophenone, 99%, propiophenone, 99%, heptanophenone, 98%, and octanophenone, 98%, were all obtained from Aldrich (Milwaukee, WI, USA), and nonaphenone was obtained from Acros Organics (Geel, Belgium). To prepare the stock solutions, 1.0 mL of each test solute was diluted to 100 mL with methanol. Analytical test solutions were obtained by diluting 1.0 mL of the stock solution to 100 mL using the corresponding mobile phase.

2.3. Procedure

Alkylphenone test solutes were studied on seven columns using an SDS mobile phase and a hydro-organic mobile phase for comparison purpose. Two lower alkylphenone homologues (acetophenone and propiophenone) were used for the Zorbax C_{18} column due to the excessive retention (>180 min at 1.5 mL/min) of the higher homologues using the 50 mM SDS mobile phase. For all other columns, the test solutes used were heptanophenone, octanophenone, and nonaphenone. The solutes were injected in duplicate for all experiments. The RPLC mobile phase consisted of a mixture of 45/55 (v/v), methanol and water for all columns. The MLC mobile phase consisted of 1-propanol–50 mM SDS (5:95, v/v) for the Zorbax C_{18}, Nucleosil C_4, Kovasil C_{14}, and FluoroSep Octyl columns. For the C_3, C_8, and C_{18} Zorbax Poroshell columns, the MLC mobile phase consisted of 1-propanol–15 mM SDS (5:95, v/v); this lower concentration of SDS was used for the Zorbax Poroshell columns because (i) experimental comparisons with 50 mM SDS showed no tangible difference in retention between the solute of interest and the t_0 marker, nitromethane; and (ii) 15 mM was the minimum concentration of SDS at which reasonable selectivity was observed.

For all experiments, UV detection at 254 nm was used, and the columns were held at a constant temperature of 40.0 °C. To construct Knox plots for each column, a variety of flow rates were used. For all columns except the Kovasil C_{14}, the flow rates employed were 0.05, 0.1, 0.2, 0.4, 0.7, 1.1, and, 1.5 mL/min. Due to pressure restrictions, the flow rate of the Kovasil C_{14} column was limited to 0.7 mL/min, and the flow rates used for it were 0.05, 0.1, 0.2, 0.35, 0.5, and 0.7 mL/min. Nitromethane was used as a t_0 marker for all experiments. The various particle, pore sizes and surface areas of the columns made it necessary to vary by column the injection volume of the test solutions. For each column, the injection volume employed was based on the manufacturers' recommendations: 40 μL for the Zorbax C_{18} and Nucleosil C_4, 20 μL for the FluoroSep Octyl, 10 μL for the Kovasil C_{14}, and 2 μL for all Poroshell columns.

The diffusion coefficients of the alkylphenones were determined using the Taylor–Aris dispersion technique [34–37]. The apparatus was modeled after that used by other workers [38–40]. A 1585-cm length 316 stainless steel tube (0.020 in. (50.8 μm) inner diameter, 0.0625 in. (159 μm) outer diameter) (Alltech Associates, Deerfield, IL, USA) was wound into 16-cm coils and placed in a constant temperature water bath at 40.0 ± 0.1 °C. The same HP1100 HPLC used in the MLC experiments described above was used to measure the diffusion coefficients. The stainless steel tubing was connected directly from the injector to the detector to eliminate any other intermediate tubing of varying length and radii. Five μL injections of each solute in its corresponding mobile phase were made in duplicate. The flow rate was maintained at 0.10 mL/min with detection at 254 nm. Provided that the flow is laminar, a Gaussian peak is obtained. For liquids, the diffusion coefficient may then be calculated from the expression [41].

$$D = \frac{0.2310 r^2 t_R}{(W_{1/2})^2} \quad (2)$$

where D is the diffusion coefficient of the solute expressed in cm^2/s, r is the radius of the capillary tube expressed in centimeters, t_R is the residence time of the solute in the tubing expressed in seconds, and $W_{1/2}$ is the peak width at its half height expressed in seconds. Secondary flow effects should be considered [38,42], but can be neglected through use of a sufficiently long column of the correct radius, flow rate, and coil diameter. The accuracy of the apparatus and conditions used were evaluated by measuring two of the test

Table 1
Measured diffusion coefficients in various solvents at 40 °C

Solute	D ($\times 10^{-6}$ cm^2/s)				
	Methanol–water (30:70)[a]	Methanol–water (30:70)	Methanol–water (45:55)	1-Propanol–0.015 M SDS (5:95)	1-Propanol–0.050 M SDS (5:95)
Acetophenone	8.84	9.46	9.39		6.82
Propiophenone			8.64		4.69
Heptanophenone	5.85	6.85	6.55	2.15	1.67
Octanophenone			6.22	1.97	1.59
Nonaphenone			5.99	1.85	1.57

[a] For comparison from ref. [40].

solutes under the same conditions as previously experimentally determined [40]. In addition, the absence of any peak abnormalities such as tailing indicated our system was reliable. Table 1 lists the values of the determined diffusion coefficients.

3. Results and discussion

Knox plots of reduced plate height versus reduced velocity are used to compare efficiencies between chromatographic systems. The Knox equation is [43]:

$$h = A'\nu^{1/3} + \frac{B'}{\nu} + C'\nu \tag{3}$$

where A', B', and C' are constants related to flow anisotropy, longitudinal diffusion, and mass transfer processes, respectively; h is the reduced plate height and ν is the reduced mobile-phase velocity. The A' term is important as it is related to the flow through the column and the band broadening due to eddy dispersion. The adsorption of surfactant on the stationary phase is related to the A' term in that the adsorbed surfactant changes the surface of the stationary phase and the micelle–stationary-phase interaction. In addition, the charge buildup of the surfactant may contribute to repulsion of the micelles and limit the ability of the micelle to penetrate the pores. The C' term represents the contributions from the various mass transfer processes: mobile-phase mass transfer, stationary-phase mass transfer, and stagnant mobile-phase mass transfer.

To calculate reduced plate height, an accurate calculation of the plate number is required. The use of either the statistical moment method [44] or the equation developed by Foley and Dorsey [45] has been shown to provide the most accurate determination of theoretical plates for non-ideal peaks. To that end, the use of statistical moments was employed here for all theoretical plate count measurements. Column efficiency using the statistical moments method is calculated by $N = M_1^2/M_2$, where N is the column efficiency, M_1 is the first statistical moment, and M_2 is the variance (second centroid moment) [44]. ChemStation software calculates the number of theoretical plates using statistical moments, as well as the zeroeth through fourth moments, which greatly facilitates the use of this approach. Where the resolution of the mixed component solutions was less than baseline, the individual components were injected in order to accurately measure N with the statistical moments method. The reduced plate height was calculated by:

$$h = \frac{H}{d_p} \tag{4}$$

where H is the plate height and d_p is the particle size of the stationary phase.

For reduced velocity, the overall diffusion coefficient must be known for each solute in each mobile phase. The reduced velocity was calculated by:

$$\upsilon = \frac{u(d_p)}{D} \tag{5}$$

where u is the superficial linear velocity of the mobile phase as determined by the retention of the t_0 marker, nitromethane.

When using Knox plots, it is generally accepted that a well-packed column is represented by a minimum reduced plate height of four or less ($\log h \leq 0.6$). In this work, we are primarily interested in demonstrating improved efficiency with micellar mobile phases over that of a hydro-organic mobile phase. Most data show that neither hydro-organic, nor micellar mobile phases demonstrated a minimum $\log h \leq 0.6$. This may be partly because the experimental conditions were selected to remain constant for all columns to allow for ease of comparison. Optimization of experimental conditions, including mobile phase and column temperature, would likely result in improved absolute efficiencies. In addition, as the given Knox plots are displayed in logarithmic scale, small differences in $\log h$, or small offsets in the Knox plot curves, represent significant differences in efficiency.

Due to the excessive retention of the higher alkylphenone homologs, it was necessary to use acetophenone and propiophenone as test solutes for the Zorbax C_{18} column. Therefore, care should be taken when making a direct comparison of the reduced parameters of this C_{18} column to the other columns. Additionally, although the same flow rates were used for all columns, the reduced velocity will be different among the columns due to the contributions of linear velocity (dependent on column inner diameter), particle size, and diffusion coefficient, as shown in Eq. (5). These differences should be considered when making comparisons among the columns. Our evaluations are primarily focused on a comparison of

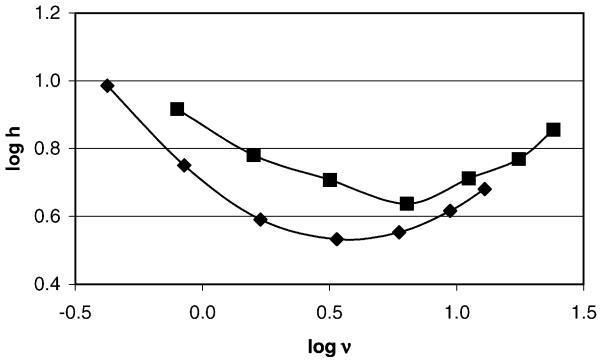

Fig. 1. Knox plot of propiophenone: Zorbax ODS in MeOH–water (45:55) (♦) and 1-propanol–50 mM SDS (5:95) (■).

the improvement (or lack thereof) in the reduced plate height for each column from the hydro-organic mobile phase to the micellar mobile phase.

Fig. 1 shows the Knox plot evaluation of the Zorbax C_{18} column with 70 Å pore size. The mass transfer contribution from the C' term is approximated by the slope of the curve at higher reduced velocities. The increase in slope indicates a higher resistance to mass transfer. Fig. 1 illustrates the reduced efficiency in MLC using the Zorbax C_{18} column with 70 Å pore size even when 1-propanol and elevated column temperatures are employed.

To determine the contribution of the intraparticle mass transfer, two approaches were taken. First, a non-porous column, Kovasil C_{14}, was used to eliminate the variance due to intraparticle mass transfer. Fig. 2 shows that the Knox plots for both the micellar and hydro-organic mobile phases do not demonstrate an increase in slope as their reduced velocities increase. This indicates that there is little resistance to mass transfer in either case. The generally flat profile of the micellar mobile phase suggests that the differences in log *h* between the micellar and hydro-organic mobile phases at lower reduced velocities may be due to increased flow anisotropy magnified by the surfactant adsorption onto the stationary phase. Although 5% 1-propanol was added to the mobile phase, a previous study by Hinze and coworkers showed that only a modest reduction in adsorbed surfactant on a C_{18} column could be expected with 5% propanol in the mobile phase, i.e., 28% less adsorbed surfactant in the previous study [8]. Since the polarities of C_{18} and C_{14} are similar, the amount of adsorbed surfactant on the C_{14} column should also be similar. Although reduced, the adsorbed surfactant may continue to affect the flow anisotropy.

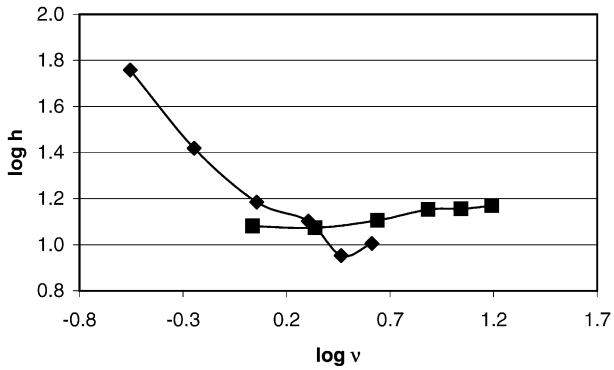

Fig. 2. Knox plot of nonaphenone: Kovasil C_{14} non-porous in MeOH–water (45:55) (♦) and 1-propanol–50 mM SDS (5:95) (■).

To further study the contribution of the variance due to intraparticle mass transfer, three superficially porous Poroshell columns were obtained from Agilent. The Poroshell particles have a solid core of silica in the center surrounded by a thin layer of 300 Å porous silica, rather than a completely porous silica particle, and their specific surface area is in between that of conventional porous bonded-phase silica and non-porous bonded-phase silica. These columns are primarily used for the analysis of proteins because their intermediate pore diameters are sufficient to allow most proteins to enter the pores while their relatively shallow pore depth is designed to minimize intraparticle mass transfer, at least in terms of the slow diffusion of large compounds in the stagnant mobile phase within the pore. We hypothesized that intraparticle mass transfer might also be significantly reduced using these columns with smaller analytes under MLC conditions, recognizing that the pore diameter of 300 Å is somewhat less than the optimal in terms of micelle penetration and the corresponding eluting strength of the mobile phase [14].

Fig. 3 a–c shows the Knox plots for nonaphenone for the C_3, C_8, and C_{18} Poroshell columns. As with the non-porous Kovasil C_{14} column, a significant improvement in the micellar efficiency was not noticed. The apparent lack of improvement in efficiency in both the non-porous and Poroshell columns indicates that the intraparticle mass transfer is not the dominant factor in the loss of efficiency. A comparison of the Knox plots of the three Poroshell columns, C_3, C_8, and C_{18}, showed that all three columns had similar MLC efficiencies, where the C_3 column was slightly improved over the C_8 and C_{18} columns in the analysis of heptanophenone and octanophenone. Conversely, the Knox plots using the hydro-organic mobile phase showed the Poroshell efficiency to be clearly improved in order of decreasing polarity of the stationary phase: $C_3 > C_8 > C_{18}$. Since the C_3 column showed the greatest net improvement in efficiency from hydro-organic to micellar mobile phase, it is assumed that this is due to the decreased adsorbed surfactant on the C_3 column as compared to the C_8 and C_{18} columns. The amount of surfactant adsorbed by a C_8 column has previously been shown to be substantially less than that adsorbed by a C_{18} column [19].

Dorsey et al. [2] suggested the use of short alkyl bonded phases as an additional means to improve efficiency in MLC. Cline-Love showed that a C_1 column had unique selectivity properties over a traditional C_{18} column. One interesting finding from Berthod et al. [19] showed that a C_1 column had the greatest amount of adsorbed surfactant as compared to C_{18}, C_8, CN, and bare silica columns. This was unexpected and they concluded that the mechanism for adsorption onto moderately polar stationary phases, like C_1, is not only due to hydrophobic interactions, but possible silanophilic inter-

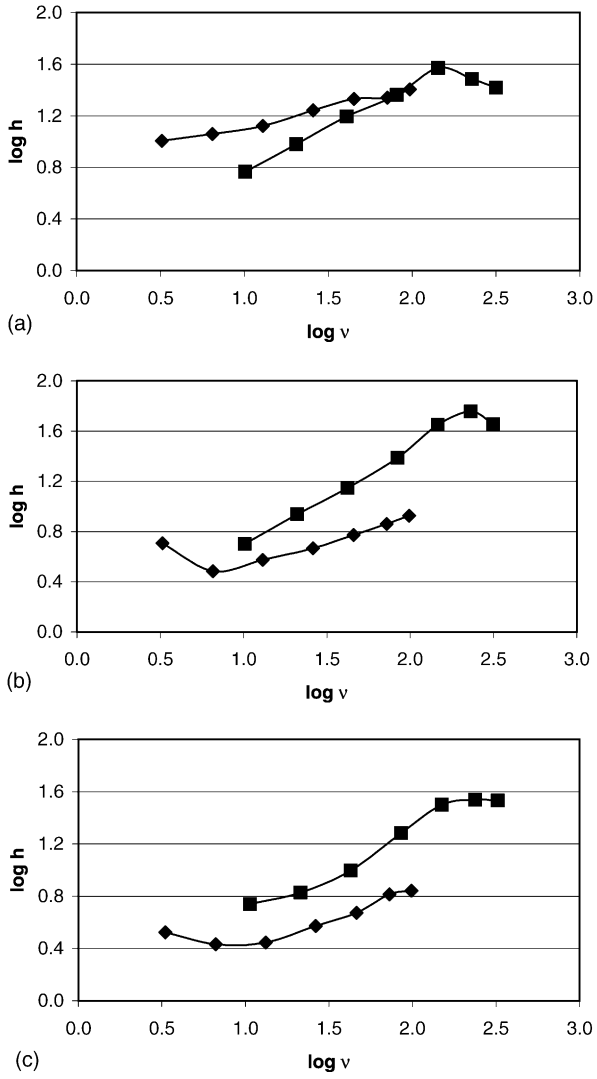

Fig. 3. Knox plots of nonaphenone: (A) Poroshell C_3 300 Å in MeOH–water (45:55) (♦) and 1-propanol–15 mM SDS (5:95) (■). (B) Knox plot of nonaphenone: Poroshell C_8 300 Å in MeOH–water (45:55) (♦) and 1-propanol–15 mM SDS (5:95) (■). (C) Knox plot of nonaphenone: Poroshell C_{18} 300 Å in MeOH–water (45:55) (♦) and 1-propanol–15 mM SDS (5:95) (■).

actions. Therefore, we chose to use a slightly less polar stationary phase, C_4, in the hopes that less surfactant adsorption would occur. Berthod did not study a C_4 column; however, the C_8 column showed the least amount of adsorbed surfactant of the columns studied. Fig. 4 clearly shows that the C_4 1000 Å stationary phase had superior efficiency with the micellar mobile phase compared to the hydro-organic mobile phase. However, the large positive slope and the absence of a discernable minimum in the micellar Knox plot may still indicate a mass transfer issue within the mobile phase.

A fluorinated column was selected to further explore the possibility that reduced adsorbed surfactant can contribute to efficiency gains. Yang et al. [46,47] first reported on the use of fluorinated stationary phases in MLC in 1994. They reported both decreased retention and improved efficiencies using a

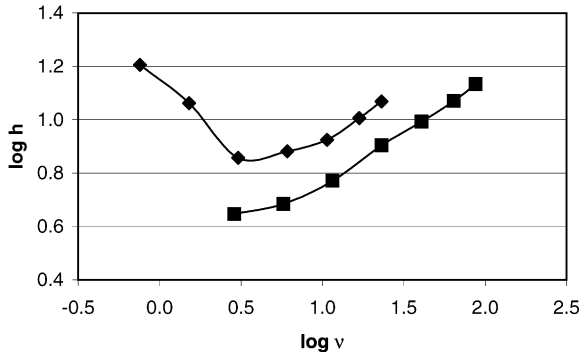

Fig. 4. Knox plot of nonaphenone: Nucleosil C_4 1000 Å in MeOH–water (45:55) (♦) and 1-propanol–50 mM SDS (5:95) (■).

standard pore size fluorooctyl (FO) column. Our experiments using a large-pore (1000 Å) FO column also show improved efficiency as well as greatly reduced retention for the higher alkylphenone homologues. Fig. 5 shows the Knox plots of micellar mobile phase are improved versus the hydro-organic mobile phase. Further, the relative flatness of the curve indicates that the resistance to mass transfer is low with respect to both intraparticle and interstitial mass transfer. According to Meyer [48], if $\log h < 1$, when $\log \nu = 2$, then the stationary phase has good mass transfer properties for the injected solute. In Fig. 5, at $\log \nu = 1.8$, $\log h = 0.82$, indicating that as $\log \nu$ approaches 2, the FO column has good mass transfer properties. Fig. 6 shows the slight improvement in peak shape using the FO column with micellar mobile phase as compared to the hydro-organic mobile phase. Fig. 7 illustrates the classical lack of efficiency with micellar mobile phase using the Poroshell C_{18} column.

The two stationary phases which showed improved efficiency over hydro-organic mobile phase were the large-pore C_4 and FO columns. This may be indicative of reduced amounts of adsorbed surfactant due to the polarity difference of the micelle and the stationary phases. Given that surfactant adsorbed onto a bonded stationary phase is believed to increase the viscosity of both the stationary phase and the interfacial regions between the stationary and mobile phases, a smaller amount of surfactant adsorption should result in a smaller reduction in mass transfer and a smaller increase in

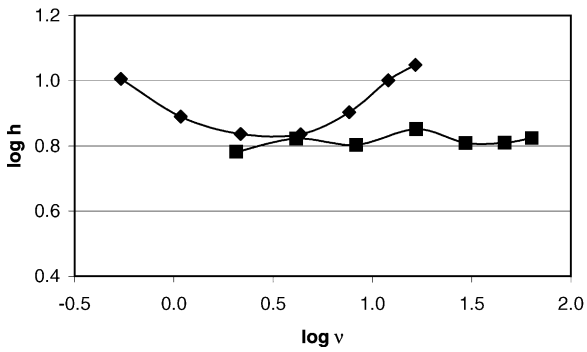

Fig. 5. Knox plot of nonaphenone: FluoroSep Octyl 1000 Å in MeOH–water (45:55) (♦) and 1-propanol–50 mM SDS (5:95) (■).

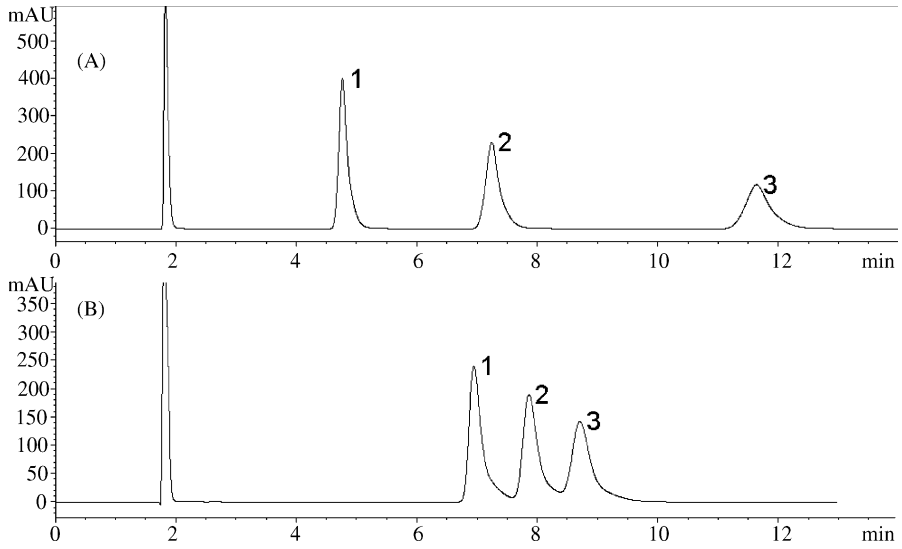

Fig. 6. Separation of (1) heptanophenone, (2) octanophenone, and (3) nonaphenone on a FluoroSep Octyl 1000 Å column at 40 °C. The flow rate used was 0.7 mL/min, λ = 254 nm, mobile phase: (A) MeOH–water (45:55), (B) 1-propanol–50 mM SDS (5:95).

flow anisotropy. This should be further confirmed by the study of adsorption isotherms of the surfactant on all the columns used.

Table 2 provides a summary of retention factor, number of plates per column, and reduced plate height for the various columns at a flow rate of 0.7 mL/min using MLC and hydro-organic mobile phases. Although this flow rate was not the optimized one as determined by the Knox plots, it represents a practical flow rate for comparison in which the retention times of all solutes were less than 20 min. As stated previously, the differences in reduced velocities are due to differences in diffusion coefficients of the analytes, as well as column particle size and column inner diameter. The reduced velocities for nonaphenone are given for comparison. All plate counts reported were calculated by the statistical moments method and are generally somewhat lower than those calculated by other means, i.e., equations based on Gaussian peak shapes [45,49].

The higher plate count of the C_4 and FO columns in MLC are noteworthy as it demonstrates that the presumed reduced adsorbed surfactant is a major contributor to improvements in efficiency in MLC. Additionally, although the Poroshell columns did not show an improvement in efficiency, the C_3 column was more efficient in MLC than the C_8 and C_{18} Poroshell columns, whereas it was the least efficient under hydro-organic conditions. This again, supports the theory that the reduced stationary-phase adsorption provides a means to improve efficiency in MLC and is in agreement with previous conclusions reached in this area by several researchers such as Borgerding et al. [8], Berthod et al. [9], and Lavine and Hendayana [26].

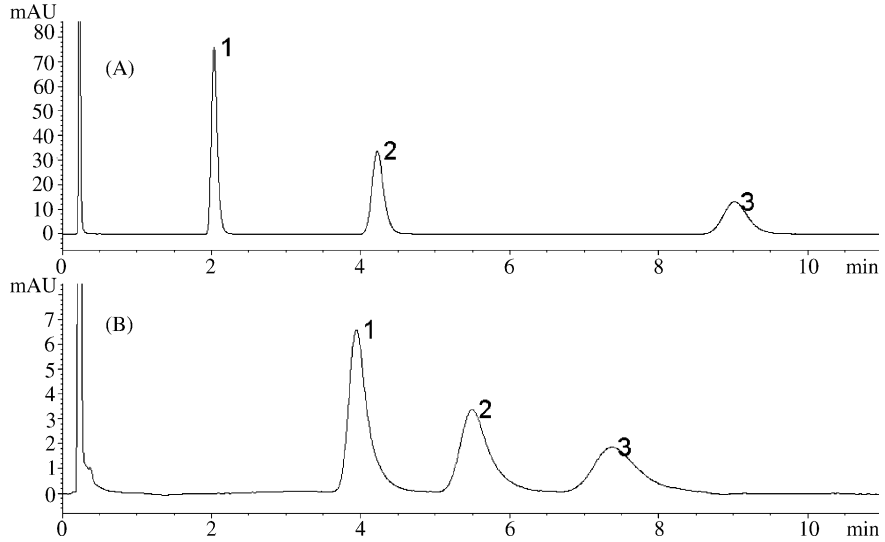

Fig. 7. Separation of (1) heptanophenone, (2) octanophenone, and (3) nonaphenone on a Poroshell C_{18} 300 Å column at 40 °C. The flow rate used was 0.7 mL/min, λ = 254 nm, mobile phase: (A) MeOH–water (45:55), (B) 1-propanol–15 mM SDS (5:95).

Table 2
Comparison of retention and efficiency in micellar and reversed-phase liquid chromatography

Column	Mobile phase[a]	Nitromethane t_R (min)	Heptanophenone			Octanophenone			Nonaphenone			
			k	N[b]	h[c]	k	ν[d]	N[b]	h[c]			
Kovasil C_{14}	0.050 M SDS	0.34	2.38	1080	20.4	3.03	1350	16.4	3.77	15.5	1490	14.8
	MeOH–water	0.34	7.39	1440	15.3	16.6	1780	12.7	36.6	4.09	2190	10.2
Poroshell C_3	0.015 M SDS	0.24	20.8	827	18.2	27.8	750	20.0	35.5	143	410	37.9
	MeOH–water	0.23	0.87	919	16.3	1.51	875	17.1	2.61	45.0	700	21.4
Poroshell C_8	0.015 M SDS	0.23	15.1	568	26.6	20.6	596	25.2	26.7	147	335	44.8
	MeOH–water	0.23	3.57	1330	11.3	7.16	2050	7.33	14.3	45.8	2540	5.91
Poroshell C_{18}	0.015 M SDS	0.22	16.6	706	21.3	23.5	648	23.2	31.9	150	474	31.7
	MeOH–water	0.23	7.99	2330	6.45	17.8	2760	5.43	39.1	46.3	3180	4.71
Nucleosil C_4	0.050 M SDS	4.58	2.00	4410	8.10	2.43	4370	8.17	2.88	40.6	3640	9.82
	MeOH–water	4.57	1.45	3880	9.21	2.68	4380	8.16	4.95	10.7	4240	8.42
FluoroSep octyl	0.050 M SDS	1.80	2.85	2740	7.29	3.36	4300	4.65	3.83	29.5	3100	6.45
	MeOH–water	1.82	1.60	2500	8.00	2.94	2580	7.77	5.34	7.64	2500	8.00

[a] Flow rate = 0.7 mL/min; SDS mobile phases contains 5% 1-propanol; MeOH–water mobile phases contain 45% methanol and 55% water.
[b] N = plates/column as calculated by statistical moments method.
[c] h = reduced plate height.
[d] ν = reduced velocity, ν for heptanophenone and octanophenone are smaller due to smaller diffusion coefficients (Table 1).

4. Conclusion

Seven stationary phases were evaluated to determine the effect of pore size and chain length on efficiency in MLC. The MLC efficiency of the commonly used C_{18} column with a conventional pore size was shown to be lower than that of hydro-organic mobile phase despite the traditional remedies of increased temperature and alcohol addition. Large-pore and non-porous columns were evaluated to determine the effect of the pore size on efficiency in MLC. Improvements in the intraparticle mass transfer strictly due to pore size were not readily observed. However, the combination of large pores and less non-polar stationary phases was shown to improve efficiency and reduce retention in MLC. The advantage of the large pore size was demonstrated by the reduced retention of normally excessively retained analytes. It is believed that the observed improvements in efficiency are due to the reduced amount of surfactant adsorbed on the C_4 and fluorooctyl columns. The smaller amount of surfactant adsorption should result in both a smaller reduction in mass transfer and a smaller increase in flow anisotropy, thus providing greater efficiencies. The results of the studies with the C_4 and FO columns show that continued improvements in efficiency in MLC can be made through the selection of stationary phases that may adsorb less surfactant. Stationary phase and pore size should be considered prior to conducting experiments in MLC to ensure optimum efficiency and retention.

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