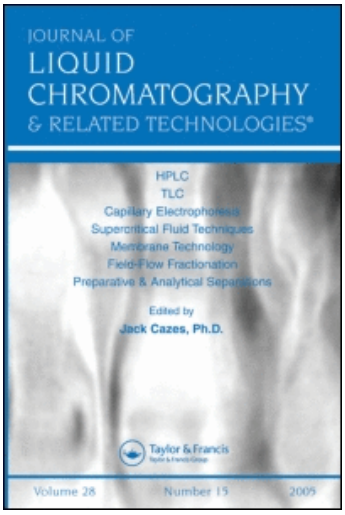

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Band Broadening in Micellar Liquid Chromatography

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BAND BROADENING IN MICELLAR LIQUID CHROMATOGRAPHY

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ABSTRACT

The effect of temperature on efficiency in micellar liquid chromatography (MLC) has been investigated using an SDS micellar mobile phase and a C_{18} stationary phase. Application of the Knox equation to plate count data yielded crucial information about band broadening in MLC. The improvement in chromatographic efficiency with temperature is due to a decrease in both the A (flow anisotropy) and C (stationary phase mass transfer) terms of the Knox equation. The decrease in the A term can be attributed to a shift in the position of the equilibrium of the solute away from the micelle and towards the bulk solvent; the decrease in the C term with temperature can be explained in terms of surfactant adsorption which tends to increase both the thickness and viscosity of the stationary phase. By increasing the operating temperature of the column, less surfactant is adsorbed on the stationary phase.

Due to concerns about the validity of the Knox equation to describe band broadening in MLC, it was necessary to consider other studies of micellar mobile phases in relation to their role in MLC, in order to obtain physically meaningful values for the A,

B, and C terms of the Knox equation. When the different equilibria involving the micelle and surfactant were taken into account, the low rate of surfactant desorption could not be ignored as the underlying cause of band broadening in MLC. If the desorption rate of surfactant molecules on an alkyl bonded phase is too low, the result is a disturbance in the various equilibria involving the micelle and surfactant monomer. This disturbance would explain the continued adsorption of SDS on the stationary phase at concentrations in excess of the critical micelle concentration of the surfactant. The low rate of surfactant desorption would also affect the dynamics of micellization which play a crucial role in MLC mobile phase mass transfer.

INTRODUCTION

In 1980, Armstrong and Henry[1] first demonstrated that aqueous micellar solutions can be used as mobile phases in reverse phase liquid chromatography (RPLC). They called this technique pseudophase or micellar liquid chromatography (MLC). Since the first report by Armstrong and Henry, the potential applications and unique capabilities of MLC have been investigated. More than one hundred papers to date including several review articles and a symposium series volume[2-7] have been published on the unique advantages of MLC.

Clearly, micellar mobile phases have certain advantages over traditional hydro-organic mobile phases in RPLC, e.g, direct injection of biologicals, resolution of optical isomers via chiral micelles, and unusual selectivity to name a few. However, there is a problem with MLC - it tends to be less efficient than conventional RPLC.

Dorsey et al[8] were the first to address this problem. They believed the reduction in column efficiency was due to slow mass transfer, which arises principally from poor wetting of the stationary phase. Dorsey demonstrated that chromatographic efficiency in MLC can be improved by adding a small amount of propanol, 3% by volume, to the mobile phase. Yarmchuk and Cline-Love,[9] on the other hand, attributed the reduced efficiency associated with ionic micellar mobile phases to poor mass transfer between the micelle and the stationary phase, with the micelle exit rate constant being the limiting factor for

hydrophobic solutes. Borgerding and Hinze[10] concluded that poor mass transfer within the stationary phase itself, resulting from adsorption of surfactant onto the alkyl bonded phase, is responsible for the low efficiencies observed in MLC. They demonstrated that addition of an alcohol, such as isopropanol, to a nonionic micellar solution reduces the amount of surfactant adsorbed on the stationary phase, resulting in a more efficient separation. In contrast to what has been reported by other workers, Cassidy[11] in a recent study on band broadening in MLC concluded that improvement in solute mass transfer which can occur upon addition of propanol to an SDS micellar solution is due to changes in the structure of the micelles, not mass transfer effects related to the loading of surfactant on the bonded phase.

Clearly, there is disagreement among workers concerning the reason for the low efficiencies evidenced in MLC. While the addition of a medium chain length alcohol such as propanol to a micellar mobile phase has been shown to improve column efficiency significantly, the presence of an alcohol in the mobile phase can affect the retention mechanism by shifting the equilibrium of the solute away from the stationary phase and the micelle and toward the bulk aqueous phase.[12] In addition, the added organic modifier can greatly complicate the interpretation of plate count data because the properties of the micelles in these so-called hybrid mobile phases are also influenced by the presence of the alcohol,[13-14] but it is not clear to what extent. Hence, there is a limit to the information that can be garnered about the underlying cause of the reduced efficiencies in MLC from experiments involving alcohol containing micellar solutions.

Therefore, a different approach, varying the operating temperature of the column, was employed in this study to better understand the causes of band broadening in MLC. Yarmchuk and Cline-Love in 1984[9] showed that increasing the operating temperature of the column in micellar RPLC enhances the efficiency of the separation process in MLC. However, Yarmchuk's study was limited to the C_1 alkyl bonded phase. Unlike a C_{18} column, the efficiency of a C_1 column does not improve significantly with the addition of an alcohol such as propanol to the micellar mobile phase. Furthermore, the unusual selectivity exhibited by C_1 bonded phase columns towards ionogenic solutes in MLC is well known to many workers in the field of micellar RPLC.[15-16] For example, the retention time of some ionogenic compounds on C_1 bonded phase columns actually increases with increasing micelle concentration, which is opposite of what is considered normal retention behavior in MLC. This effect will occur with compounds that possess the same charge as the surfactant and is

considered to be an excluded volume effect, because the compound is excluded not only from the micelle, but from the double layer that surrounds the micelle. Since this effect is not observed with C_{18} and C_8 alkyl bonded phases, the so-called antibinding behavior probably occurs as the result of a different form of surfactant monomer association with the methyl bonded phase, which implies that C_{18} and C_1 bonded phase columns interact very differently with ionic surfactants. Although some workers[15-16] have argued that antibinding behavior occurs on C_1 columns as a result of the methyl stationary phase not adsorbing appreciable amounts of surfactant, Berthod[17] has in fact shown that ionic surfactants, such as SDS and CTAB, exhibit maximum adsorption on C_1, not C_{18} or C_8 alkyl bonded phases.

Because the effect of temperature on surfactant adsorption and micellar structure[18-20] is reasonably well understood, we believe that re-examining the relationship between column temperature and efficiency in MLC with a C_{18} bonded phase column can yield additional information and insight into the causes of band broadening in MLC for stationary phases with serious wetting problems. In this paper, we present the results of a comprehensive study on the effects of temperature, and flow rate on efficiency in MLC using an SDS micellar mobile phase and a C_{18} alkyl bonded stationary phase.

EXPERIMENTAL

1. HPLC System

All high performance liquid chromatographic (HPLC) measurements were made with a Rainin 81-20 M analytical HPLC system which incorporated two Rainin Rabbit HP pumps (Rainin Instruments, Woburn, MA), an Apple MacIntosh computer as the controller and data station, a Model 7125 Rheodyne injection valve with a 30 μL loop (Cotati, CA), and a Rainin Dynamax Mixer. The detector was a variable wavelength Knauer UV/Visible spectrometer (West Berlin, Germany). The extra column volume of the system was less than 60 μL.

The analytical column was an Apex I 5-μm octyldecyldimethyl silane ODS (100 mm x 4.6 mm, Jones Chromatography, Lakewood, CO). A silica guard column placed between the injector and the pump saturated the mobile phase with silicates, minimizing dissolution of the column packing. Both the

analytical column and mobile phase reservoir were water-jacketed and temperature controlled with a Haake (Berlin, Germany) circulator. The dead volume of the column was determined by injecting different solutions such as methanol, methanol-water, or water onto the columns. Dead volume measurements obtained for micellar mobile phases were comparable to the values obtained for methanol-water mobile phases. This volume, approximately 1.00 mL, was used in all k' calculations. The k' values reported in this study were averages of at least triplicate determinations.

2. Materials

Sodium dodecyl sulfate (SDS) was obtained from BDH Chemicals and was purified prior to use by first dissolving it in ethanol followed by addition of charcoal to the solution. The charcoal was then separated from the mother liquor via filtration, and the SDS was recrystallized from the mother liquor. The test solutes which were obtained from Aldrich and Sigma were used as received. Stock solutions of the test solutes were prepared in methanol and diluted to the appropriate working concentration with 0.05 M SDS for the micellar system or 30% methanol in water for the hydro-organic system. Working concentrations of injected solutes were; acetophenone (57 μg/mL), benzene (40 μg/mL), nitrobenzene (55 μg/mL), methylbenzoate (65 μg/mL), toluene (45 μg/mL), p-nitrophenol (60 μg/mL), phenol (45 μg/mL), and coumarin (30 μg/mL).

The SDS micellar mobile phase solutions were prepared by dissolving the appropriate amount of surfactant in HPLC grade water and filtering the solution twice with a 0.45 μm Nylon membrane filter. All mobile phase solutions were prepared using HPLC grade solvents which were degassed prior to use. pH measurements were made on these solutions using a Chem Trix pH meter. The pH of each solution was approximately 7.3. For the test solute p-nitrophenol (pKa = 7.1), the pH of the mobile phase was adjusted to 3.0 prior to use to prevent deprotonation of the phenol.

3. Procedure

The analytical column was not considered to be equilibrated with the mobile phase unless the retention times were constant. Chromatograms were obtained with mobile phase flow rates varying from 0.1 mL to 3.5 mL/minute.

Flow rates were measured by collecting the effluent in a 10 mL graduated cylinder for a sufficient length of time to ensure collection of at least 7 mL. The effect of temperature on efficiency in MLC and conventional RPLC was investigated using the following mobile phases: 0.02M SDS, 0.05M SDS, and a solution of 30% methanol in water. The Foley-Dorsey method[21] was used to compute the number of theoretical plates . Although there are many methods available for the calculation of chromatographic efficiency, Bildingmeyer and Warren[22] have shown that it is the most accurate manual method for plate count calculation. Berthod[23] also found this to be true. Because the Foley-Dorsey equation for plate count corrects for the asymmetry in skewed peaks, reliable chromatographic figures of merit can be obtained from tailing peaks using this method.

The Knox equation[24] was used in this study to assess the contributions of flow anisotropy, longitudinal diffusion, and other band broadening processes to the final peak bandwidth. This equation is the most widely accepted plate height equation in chromatography and can be expressed in the following form

$$h = Av^{1/3} + B/v + Cv \qquad (1)$$

where A, B, and C are the constants of the Knox equation, h is the reduced plate height ($h = H/d_p$, where d_p is the stationary phase particle diameter, and H is the column plate height), and v is the reduced mobile phase velocity ($v = ud_p/D_m$, where u is the mobile phase linear velocity (cm/sec) and D_m is the solute mobile phase diffusion coefficient). H was computed from the plate count data as an average of triplicate determinations. Values for the A, B, and C terms of the Knox equation were obtained by a nonlinear least squares fitting of the data using the Levenburg-Marquardt algorithm. Each regression analysis was examined by influence statistics to verify the fidelity of the results which is a legitimate concern when analyzing noisy collinear data.

RESULTS AND DISCUSSION

A series of chromatograms were run to illustrate the advantages of higher operating temperatures for micellar mobile phases. Figure 1 shows the separation of a five-component test mixture on a C_{18} column using a 30% methanol/70% water mobile phase. Figure 2 shows the separation of the same test mixture using a 0.05M SDS mobile phase at 25^0 and 45^0 C. For the 0.05M SDS mobile phase, increasing the operating temperature of the system

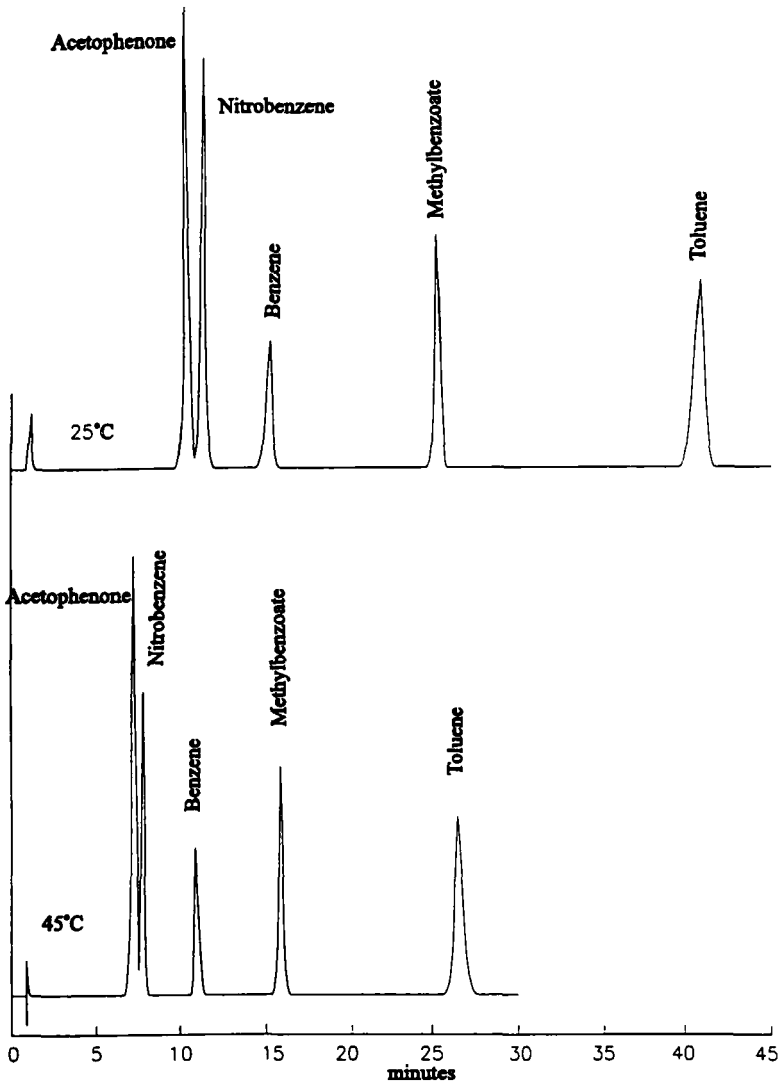

Figure 1. Chromatograms of the test mixture at 25^0 and 45^0 C for the 30% methanol in water mobile phase. An Apex I C-18 column (10 cm, 5micron particle size) was used; the flow rate of the mobile phase was 1.0 mL/min. A = nitrobenzene, B = acetophenone, C = Benzene, D = methylbenzoate, and E = toluene.

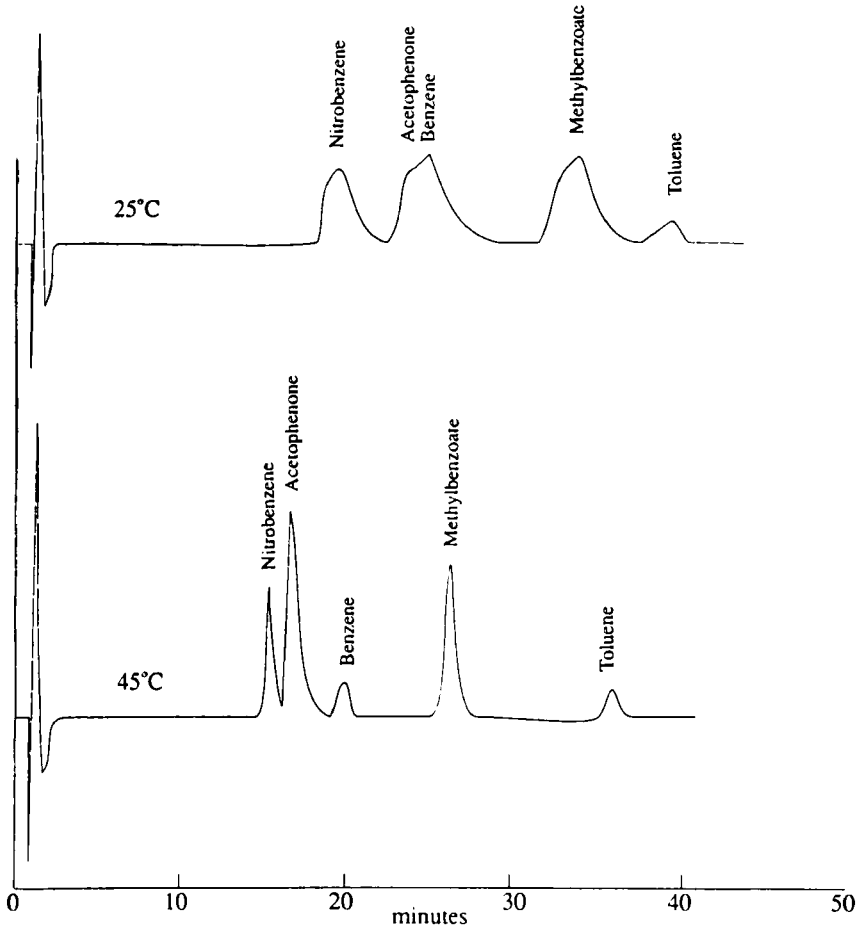

Figure 2. Chromatograms of the test mixture at 25^0 and 45^0C for a 0.05M SDS mobile phase. A = nitrobenzene, B = acetophenone, C = Benzene, D = methyl benzoate, and E = toluene.

improved both the resolution and efficiency of the separation. For the methanol in water mobile phase, there was little improvement in either resolution or efficiency.

In a simple reversed phase separation, there is initially a modest increase in efficiency due to a lowering of the mobile phase viscosity as the

operating temperature is increased. However, heat dissipation[25] is a problem in conventional reversed phase columns (e.g., particle size 5 microns, 4.6 mm id, pressure drop of a few hundred bar, flow rates of 2mL/min or greater). The inner core of the packed bed can be warmer than the wall region by 0.5 to several degrees Centigrade, with the overall result that a radial temperature profile is generated within the column which can cause non-uniformity in the migration velocity of the solute. Hence, at very high column temperatures (ca. 55 degrees Centigrade), N levels off and then decreases because of thermal gradients within the column.[26]

Table 1

Variation of Efficiency and Asymmetry with Temperature for the Test Mixture *Solutes. The Mobile Phase was 30% Methanol in Water.

	25°C		35°C		45°C		55°C		65°C		**73°C	
Compounds	**N**	**B/A**	**N**	**B/A**	**N**	**B/A**	**N**	**B/A**	**N**	**B/A**	**N**	**B/A**
Nitrobenzene	3900 (k'=10.4)	1.2	4300 (k'=7.9)	1.3	4200 (k'=6.5)	1.3	4700 (k'=5.4)	1.2	3900 (k'=4.6)	1.3	3200 (k'=3.9)	1.4
Acetophenone	3500 (k'=9.2)	1.3	4200 (k'=7.2)	1.1	4100 (k'=5.9)	1.2	4800 (k'=5.0)	1.2	3200 (k'=4.2)	1.2	2800 (k'=3.6)	1.3
Benzene	4100 (k'=14.5)	1.3	4500 (k'=11.7)	1.0	4800 (k'=9.8)	1.0	5100 (k'=8.3)	1.0	5000 (k'=7.0)	1.1	----------	
Methylbenzoate	4800 (k'=25.2)	1.1	4700 (k'=18.5)	1.3	4100 (k'=14.6)	1.2	5300 (k'=11.7)	1.3	4600 (k'=9.5)	1.2	----------	
Toluene	5100 (k'=40.9)	1.1	4900 (k'=31.8)	1.0	5100 (k'=25.5)	1.0	5400 (k'=20.8)	1.1	4900 (k'=17.0)	1.0	4700 (k'=14.5)	1.0
***p-Nitrophenol	4300 (k'=11.4)	1.3	4000 (k'=8.0)	1.3	4200 (k'=6.1)	1.2	4000 (k'=4.7)	1.3	3000 (k'=3.6)	1.4	----------	

*ODS column

**Only a few experiments were performed at 73°C because of our concern about damage to hydrolysis of the bonded phase.

***For p-nitrophenol a 20% methanol in water mobile phase with the pH adjusted to 3.0 was used because the capacity factor value of the arene was less than 5 in 30% methanol in water at 25°C.

The relationship between temperature and efficiency for the hydro-organic mobile phase (see Table 1) adheres to the simple model described above. However, the large increase in efficiency with temperature for the micellar mobile phase (see Table 2), which is greater than 100% for most of the solutes chromatographed, cannot be explained by this model, which suggests that different processes are responsible for band broadening in MLC and are also responsible for the dramatic improvement in efficiency at elevated temperatures in SDS micelle mediated liquid chromatography.

Table 2

Variation of Efficiency and Asymmetry with Temperature for the Test Mixture *Solutes. The Mobile Phase was 0.05M SDS.

Compounds	25°C N	B/A	35°C N	B/A	45°C N	B/A	55°C N	B/A	65°C N	B/A	**73°C N	B/A
Nitrobenzene	310 (k'=15.8)	3.5	900 (k'=13.6)	2.4	1600 (k'=12.2)	2.8	2300 (k'=10.7)	2.4	2700 (k'=9.3)	2.0	4200 (k'=8.9)	1.1
Acetophenone	280 (k'=19.4)	2.5	400 (k'=16.0)	3.4	980 (k'=13.6)	2.4	1800 (k'=11.2)	1.0	2100 (k'=9.8)	1.0	3300 (k'=9.5)	1.5
Benzene	1300 (k'=17.8)	2.0	1800 (k'=17.0)	1.3	3300 (k'=16.3)	1.6	4500 (k'=15.2)	1.4	4500 (k'=13.7)	1.2	----------	
Methylbenzoate	570 (k'=25.9)	2.1	900 (k'=24.1)	2.4	2000 (k'=21.4)	2.6	2800 (k'=19.1)	2.7	4700 (k'=16.8)	1.2	----------	
Toluene	2000 (k'=33.0)	1.8	2500 (k'=31.6)	1.7	3100 (k'=30.6)	1.0	3300 (k'=28.4)	1.0	3900 (k'=26.1)	1.1	5900 (k'=25.5)	1.1
***p-Nitrophenol	2600 (k'=9.2)	2.1	3300 (k'=8.7)	2.2	4900 (k'=7.6)	1.2	4500 (k'=5.8)	1.4	5300 (k'=4.9)	1.2	----------	

*ODS column

**Only a few experiments were performed at 73°C because of our concern about damage to the column due to hydrolysis of the bonded phase.

***For p-nitrophenol, a 0.02M SDS solution with the pH adjusted to 3.0 was used as the mobile phase because the k' value of the arene was less than 5 with 0.05M SDS at 25°C. On some C_{18} columns, p-nitrohphenol exhibited a split peak with the 0.02M SDS mobile phase at column temperatures in excess of 35°C. Evidently, p-nitrophenol is sensitive to changes in the structure of the C_{18} which occur as a consequence of hydrolysis of the bonded phase, a process catalyzed by elevated temperature and low pH.

1. Knox Plots

To better understand the nature of the relationship between temperature and efficiency in MLC, the Knox equation was used to study band broadening at different temperatures for two micellar mobile phase, a 0.02M SDS and a 0.05M SDS. Because of concern for the effect of viscous heat dissipation which can obscure the interpretation of HETP curves at temperatures above 45 degrees Centigrade,[26] only solutes which exhibited large increases in N with temperature, at or below 45 degrees Centigrade, were used in the Knox plot studies. The diffusion coefficient (D) of each test solute (see Table 3) was obtained from the literature[27,28] and represented a weighted average of free and micelle bound solubilizate, since the Taylor dispersion technique[29] was used in these referenced studies to determine D.

Table 3

Diffusion Coefficients of Selected Arenes.

Solute	Mobile Phase	Diffusion Coefficient (x 10^6 cm^2/sec)		
		25^0C	35^0C*	45^0C*
p-nitrophenol	0.02M SDS	4.35**	5.56	6.93
Benzene	0.05M SDS	6.80***	8.70	10.8

*The diffusion coefficient (D) at 35 and 45 degrees Centigrade was computed using the value of D at 25^0C and Waldens rule: $D_x = D_{298}$ $[T_x/T_{298}]$ $[e_{298}/e_x]$, where x is equal to 308^0K (35^0C) or 318^0K (45^0C) and e is the viscosity of the bulk solvent, water, obtained from the CRC Handbook.

**from reference 22. The Taylor dispersion method was used to measure the diffusion coefficient of p-nitrophenol in 0.02M SDS.

***from reference 23. The Taylor dispersion method was used to measure the diffusion coefficient of benzene in 0.05M SDS.

Figure 3 is a plot of reduced plate height versus reduced mobile phase velocity for p-nitrophenol at 25^0, 35^0, and 45^0 C on a C_{18} column with a 0.02M SDS mobile phase at pH=3. Table 4 lists the A, B, and C parameters of the Knox equation at these three temperatures. The noticeable improvement in

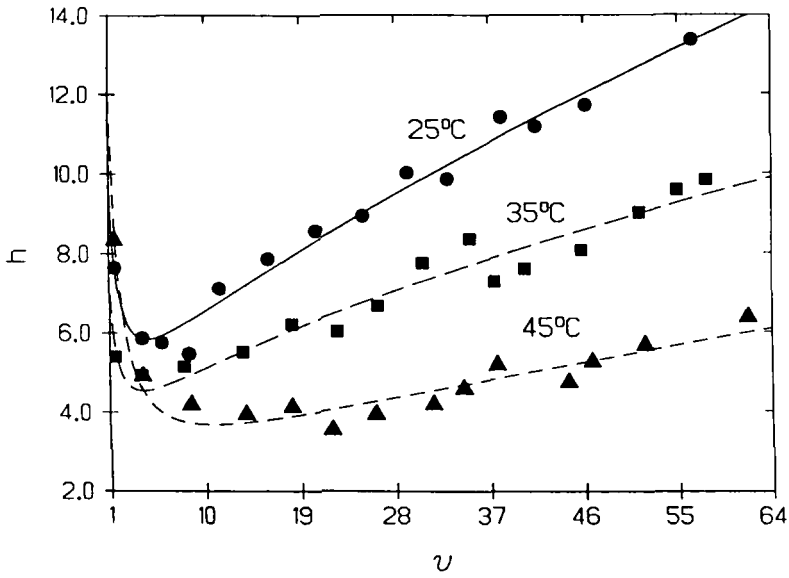

Figure 3. Reduced plate height versus reduced mobile phase velocity for p-nitrophenol on C-18 with a 0.02M SDS mobile phase at 25°, 35°, and 45°C. Each data point in the plot is an average of triplicate determinations.

chromatographic efficiency with temperature is due to a decrease in both the A (flow anisotropy) and C (stationary phase mass transfer) terms of the Knox equation. By comparison, in conventional RPLC there is generally an increase in the C term with temperature,[30] which further reinforces the conclusion that different processes are responsible for band broadening in MLC.

The decrease in the A term with temperature for p-nitrophenol can be explained by the change in the equilibrium constant for the solute between the micellar aggregate and the bulk solvent, i.e., K_2.[12] As temperature is increased, K_2 decreases, and the distribution of solute between the micelle and bulk solvent is altered. The result is that less solute is bound to the SDS micelle in the mobile phase as the operating temperature of the column is increased. Since it is easier for an arene to transfer between different solvent flow streams within the column when it exists as free solute in water instead of a bound species, the net result is that a decrease in eddy diffusion occurs which in all likelihood is responsible for the decrease in the A term of p-nitrophenol.

Table 4

Knox Equation Parameters* for p-Nitrophenol and Benzene at Various Temperatures for a 0.02M and 0.05M SDS Mobile Phase.

p-Nitrophenol (0.02M SDS)

Temperature	A	B	C	R^2
25°C	2.34(±0.14)	7.43(±0.69)	0.074(±.013)	0.99
35°C	1.96(±0.14)	5.16(±0.66)	0.030(±.011)	0.99
45°C	1.06(±0.13)	11.4(±0.65)	0.026(±.010)	0.99

Benzene (0.05M SDS)

Temperature	A	B	C	R^2
25°C	3.4(±1.1)	5.57(±2.2)	0.16(±0.22)	0.93
45°C	2.7(±0.23)	11.4(±0.54)	0.05(±.04)	0.99

*The uncertainty in A, B, and C was determined from the statistical parameters of the least squares fitting.

The decrease in the C term with temperature for p-nitrophenol can be explained in terms of surfactant adsorption which tends to increase both the thickness and viscosity of the stationary phase.[31-33] By increasing the operating temperature of the column and mobile phase, less surfactant is adsorbed on the stationary phase.[34] The rate of solute mass transfer between the mobile and stationary phase is also increased. In addition, the fluidity of the tethered alkyl chains and imbibed surfactant should increase with temperature which will also lessen stationary phase mass transfer resistance. The overall result is an improvement in column efficiency.

The large increase in the B term of the Knox equation with temperature (from 25°C vs 45°C) is due to a decrease in the viscosity of the bulk solvent. There is also a shift in the position of the equilibrium of the solute away from the micelle and towards the bulk solvent[9] as temperature is increased, also resulting in an increase in the diffusion rate of the solute because the rate of molecular diffusion is greater for free solubilizate than bound solubilizate. However, the decrease in the value of the B term of p-

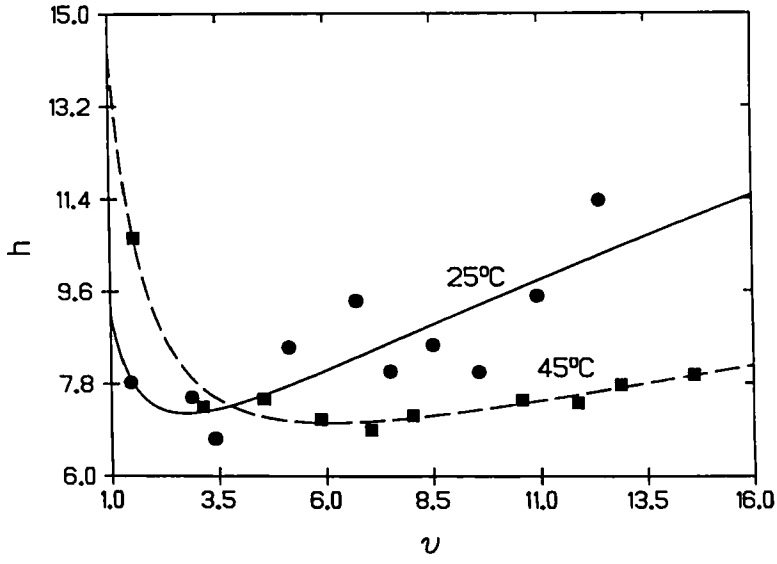

Figure 4. Reduced plate height versus reduced mobile phase velocity for benzene on C-18 with a 0.05M SDS mobile phase at 25° and 45°C. Each data point in the plot is an average of triplicate determinations.

nitrophenol as column temperature is increased from 25° to 35° C cannot be explained in terms of either a change in viscosity or a shift in the position of the micelle solute equilibrium and is probably an anomaly unique to p-nitrophenol. Because the influence statistics show the regression equation is not overwhelmed by instability, we do not believe this result is a statistical artifact arising from poorly fitted data.

In Figure 4, a plot of reduced plate height versus reduced mobile phase velocity is shown for benzene at 25° and 45° C on a C_{18} column with an unbuffered 0.05M SDS mobile phase. Table 4 lists the A, B, and C parameters of the Knox equation at 25° and 45° C for benzene. Again, the improvement in chromatographic efficiency with temperature is due to a decrease in both the A and C terms of the Knox equation. Interestingly enough, Berthod and Hinze[28] also reported that reduced chromatographic efficiencies in MLC can be attributed to large increases in both the A and C terms of the Knox equation.

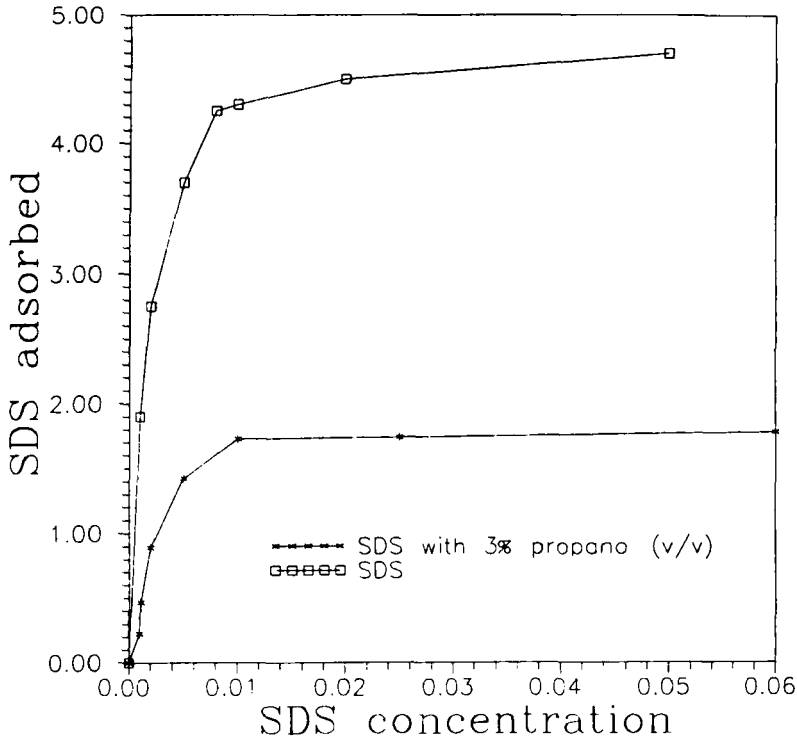

Figure 5. Adsorption isotherms of SDS on C-18 with and without propanol in the mobile phase. (micromoles/meter-squared vs. molarity of SDS). Adsorption data were obtained by pumping the appropriate concentration of surfactant in the mobile phase through the column (data from references 17 and 44).

2. Kinetics of Surfactant Desorption

Although the fit of the flow rate data to the Knox equation was good, there are concerns about the validity of this model to describe band broadening in MLC,[34] which is not surprising because the Knox equation is based on experimental data and correlations found to hold true in both packed column gas chromatography and conventional RPLC. However, MLC differs from gas chromatography or conventional RPLC due to the secondary chemical equilibria, so it is possible that one cannot model explicitly all of the salient features of band broadening in micellar RPLC using the Knox equation. Clearly, studies of micellar mobile phases in relation to their role in MLC are crucial for obtaining physically meaningful values for the A, B, and C terms of

the Knox equation. Therefore, we examined the adsorption isotherm of SDS on C_{18} to better understand the meaning of the changes in the A, B, and C terms of the Knox equation with temperature. In Figure 5, the adsorption isotherm of SDS on C_{18} at 25°C is shown. The data used in this figure was obtained by Berthod and coworkers.[17] An examination of the figure reveals a very interesting result: SDS continues to adsorb on the stationary phase at concentrations in excess of the cmc of the surfactant. This behavior is surprising in view of the fact that once the concentration of surfactant in the mobile phase exceeds the cmc, all of the added surfactant monomer should be micellized. In other words, this plot should show a cessation in the adsorption of SDS at concentrations in excess of the cmc. However, if the system is not at equilibrium, we will not see a cessation in SDS adsorption.

In micellar RPLC, the surfactant is involved in three distinct equilibria. First, there is an exchange of surfactant monomer between the micelle and the bulk aqueous phase.[35-37] This process is very fast and is characterized by a relaxation time, t_1, which is in the microsecond range. The second equilibrium involves the break-up and reformation of the micelle.[38-40] The kinetics of micellar dissociation are very complex and are characterized by a relaxation time, t_2, which is in the millisecond range. Finally, the surfactant monomer in the mobile phase is in equilibrium with molecules of surfactant adsorbed on the stationary phase. Although there is no published data on the rate of SDS desorption on hydrophobic silica, SDS desorption rates on solid surfaces such as carbon black or nylon[41-42] are found to be quite low.

In all likelihood, neither t_1 nor t_2 are responsible for band broadening in MLC. Because the exit rate constant of an SDS molecule from an SDS micelle is about 10^7 s^{-1} and the micelle surfactant association rate is nearly diffusion controlled, it is reasonable for one to assume that t_1 processes are instantaneous on a RPLC time scale. If t_2 (the stability or average lifetime of a micelle) influenced efficiency in MLC, then t_2 and chromatographic efficiency should share a similar relationship with SDS concentration. Clearly, this is not the case (see Figure 6). The average life-time of an SDS micelle increases with increasing SDS concentration up to 0.20 M SDS and then decreases.[43] However, column efficiency in MLC is observed to decrease with increasing SDS concentration, which is probably due to an increase in the viscosity of these mobile phases. Therefore, the reduction in efficiency associated with micellar mobile phases cannot be directly attributed to the dissociation kinetics of the micelle.

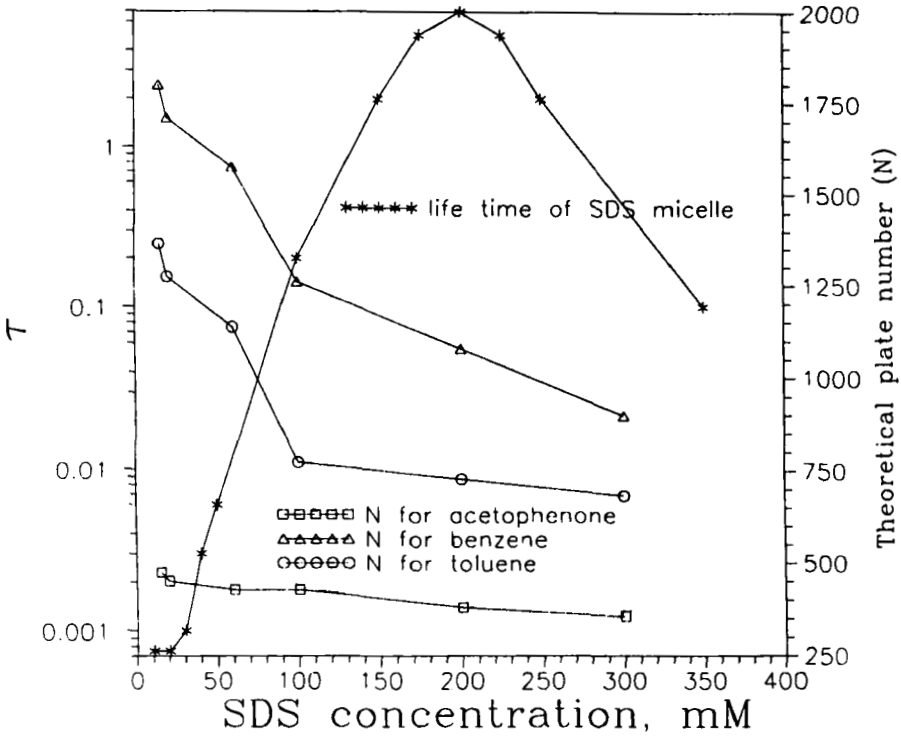

Figure 6. N and τ_2 versus concentration of SDS. Plate count data were obtained on an Apex I C-18 column; the flow rate of the mobile phase was 1.0 mL/min. τ_2 data were obtained from reference 43.

If the desorption rate of surfactant molecules on the stationary phase is too low, the result is a disturbance in the various equilibria involving the micelle and the surfactant monomer (see Figure 7). This disturbance would explain the continued adsorption of SDS on the stationary phase at concentrations in excess of the cmc. The low rate of surfactant desorption would also affect the dynamics of micellization which play a very important role in mobile phase mass transfer within the column. Assuming rapidly established equilibria between the micelle and surfactant monomer in the mobile phase and between the solute in the bulk solvent (water) and the micelle, the rate of establishment of the equilibrium between surfactant monomer in the mobile and stationary phase is then rate determining in MLC. If the rate is too slow, solute mass transfer between the mobile and stationary

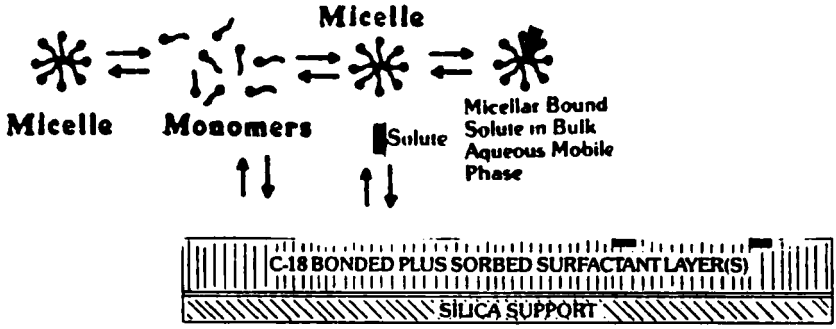

Figure 7. Equilibria in micellar reversed phase liquid chromatography. (Figure adapted from references 10 & 43.)

phases is sluggish resulting in significant broadening of the chromatographic peaks. Hence, one cannot ignore the possibility that in MLC the underlying cause of the inefficiency is the low rate of surfactant desorption which would explain the disagreement among workers concerning the reason for the low efficiencies evidenced in MLC.

3. Organic Modifiers vs Temperature

When propanol is added to an SDS micellar mobile phase (3% by volume), the adsorption of SDS on C_{18}[44] ceases after 10 millimolar SDS (see Figure 5) which is close to the cmc of the surfactant. The break observed in the SDS adsorption isotherm suggests that adsorbed surfactant monomer and the SDS micelles are at equilibrium within the column. Probably, surfactant desorption is occurring at a faster rate when a so-called hybrid mobile phase is used, which could explain the difference in shape between the adsorption isotherm of SDS and SDS hybrid micellar mobile phases. The increase in the surfactant desorption rate could also explain why efficiencies approaching those of conventional RPLC are obtained with these mobile phases, e.g., 0.05 M SDS with 3% propanol v/v.

Scott and Simpson[45] have shown that propanol preconcentrates at the C_{18} bonded phase/mobile phase interface. In other words, over 90% of the C_{18} stationary phase is covered by propanol at a concentration of 3% w/v. The preconcentration of propanol at the stationary phase probably causes the C_{18}

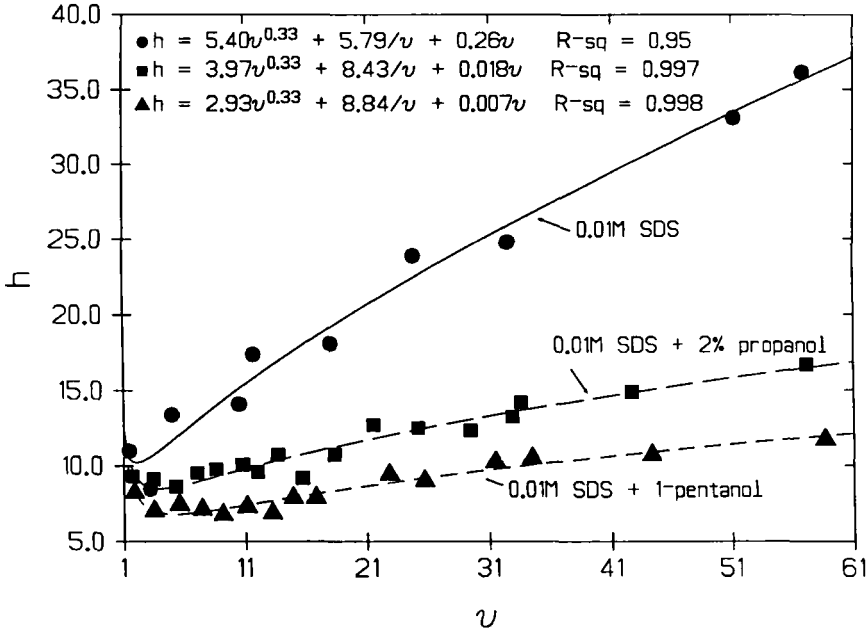

Figure 8. Reduced plate height versus reduced mobile phase velocity at 25^0 for coumarin on Apex I C-18 with a 0.01M SDS mobile phase with and without organic modifier. Each data point shown in the plot is an average of triplicate determinations.

chains to tilt toward the surface normal of the bonded phase,[46] which could explain why desorption kinetics of SDS would be more favorable on propanol modified C_{18}. In the absence of propanol, the C_{18} chains are probably tilted away from the surface normal. The net result is a less liquid-like stationary phase.[46]

The models hypothesized by researchers in microemulsion formation, in which low levels of moderate chain length alcohols are believed to enhance the flexibility of the surfactant film separating the aqueous hydrocarbon domains[47] could also be applicable to this system since the tethered C_{18} chains are analogous to an interfacial surfactant film.

Preliminary studies carried out in our laboratory on the effects of alcohol mobile phase modifiers on efficiency in MLC have shown that addition of a small amount of propanol or pentanol to a micellar mobile phase yields

improved efficiencies in MLC because of a large decrease in both the A and C terms of the Knox equation (see Figure 8), which suggests that both temperature and alcohol additives such as propanol or pentanol enhance the efficiency of the MLC separation process in much the same manner. Interestingly enough, the increase in the B term of coumarin as a result of the addition of propanol or pentanol to the micellar mobile phase can also be attributed to a decrease in the viscosity of the mobile phase and a shift in the position of the equilibrium of the solute away from the micelle and towards the bulk solvent.[12]

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