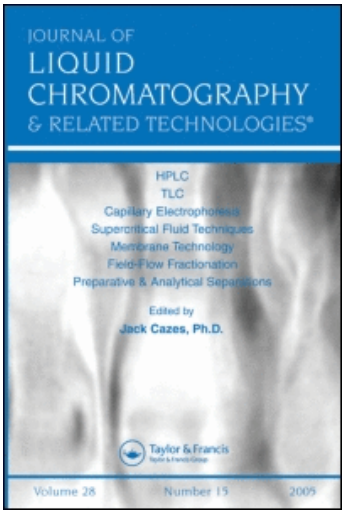

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INFLUENCE OF SEVERAL PARAMETERS ON EFFICIENCY AND PEAK SHAPE IN THE MICELLAR LIQUID CHROMATOGRAPHY OF POLYNUCLEAR AROMATIC HYDROCARBONS

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ABSTRACT

The influence of different factors on efficiency and peak asymmetry for a wide group of PAHs in micellar liquid chromatography (MLC) is studied. One can see that efficiency increases as the concentration of surfactant in the mobile phase increases. The addition of 2-propanol increases efficiency up to a 15%. The simultaneous effect of temperature and flow rate show the influence of hydrophobicity of solutes on efficiency. H-μ plots show a different behavior of PAHs in MLC as compared to RP-HPLC.

INTRODUCTION

The development of Micellar Liquid Chromatography (MLC) has experienced a solid growth in recent years. Since the first report by Armstrong and Henry[1], most of the work has been focused on exploring the advantages of

this technique.[2-12] It offers the advantages of the feasibility of optimizing parameters due to the linear retention behavior, the capability of simultaneous separation of ionic and nonionic compounds, performing gradient elution without a need for column reequilibration, gradient compatibility with electrochemical detection, direct injection of biological fluid (serum and/or plasma), usefulness in quantitative structure-biological activity relationship (QSAR studies), etc. These capabilities combined with the low toxicity and low cost of these mobile phases provide compelling reasons to consider MLC as a strong alternative to reverse phase liquid chromatography (RP-HPLC) with hydro-organic mobile phases.

The most noticeable drawback of MLC are, on the one hand, the slow resistance to mass transfer from the surfactant-modified stationary phase which leads to a poor efficiency and, on the other hand, the weak solvent strength of purely micellar mobile phases. Dorsey et al.[13] have reported that the addition of a small amount of propanol and raising the temperature to 40°C would improve the column efficiency. Yarmchuk et al.[14] have reported that poor efficiency is due to slow mass transfer of solute from micelles as well as from stationary phase to the bulk solvent. Armstrong et al.[15] noted that poor efficiency is due to a poor transfer from the surfactant modified stationary phase. All these authors suggested that chromatographic efficiency is improved with the addition of organic modifier until a determined percentage, which does not bring the system closer to a hydro-organic system, and increasing the operating temperature.

When using micellar phases in RP-HPLC the solute may interact with both the stationary and the mobile phases and thus partition equilibria are established between water and stationary phase (P_{sw}), between water and micelles (P_{mw}) and between micelles and stationary phases (P_{sm}). It is also commonly understood that only two of these three partition coefficients are necessary for a complete description of the solutes in the micelles. However, the retention mechanisms in MLC are not yet well known. Surfactant molecules adsorb on bonded stationary phases, altering the properties of the stationary phases. The addition of an alcohol modifies the surfactant adsorption, producing an alteration in the selectivity of the stationary phase as a function of the percentage and type of alcoholic modifier.[11]

Although the effect of organic modifiers on chromatographic efficiency[13] and the effect of temperature on selectivity separation[16] in MLC has been studied, the simultaneous effect of the presence of organic modifier and of temperature on efficiency and selectivity of separation has not been reported yet.

In this paper we report the results of the effect of adding alcohols to micellar eluents on the chromatographic efficiency of a series of PAHs of environmental concern. In this way the effect of the nature and percentage of alcohol as well as the influence of the temperature on the chromatographic efficiency in MLC are studied with the aim of developing separations with optimum chromatographic efficiencies in the presence of micelles of sodium dodecyl sulfate.

EXPERIMENTAL

Apparatus

All high-performance liquid chromatographic measurements were made with a Waters 600 Multisolvent Delivery System, equipped with a U6K sample injector, and a Waters Lambda-Max 481 LC variable wavelength spectrophotometric detector operating at 254 nm. Data collection and processing were provided by a Baseline 810 Waters Chromatography Workstation. The analytical column was a Waters Nova-Pak C_{18}, 150 x 3.9 mm i.d., 4 μm particle diameter. A silica precolumn was used to saturate the mobile phase with silicate to protect the analytical column and to avoid hydrolysis of the bonded stationary phase. The analytical column and the mobile phase reservoir were water-jacketed and temperature controlled with a circulating bath.

Reagents

The surfactant sodium dodecylsulfate (SDS) was of electrophoresis grade obtained from Aldrich and used as received. Methanol (MeOH), ethanol, 2-propanol (PrOH) and n-butanol (BuOH) were Merck, pro analysi products. Naphthalene (1), acenaphthylene (2), fluorene (3), anthracene (4), phenanthrene (5), 9-methylanthracene (6), fluoranthene (7), pyrene (8), chrysene (9), benzo[*b*]fluoranthene (10), benzo[*a*]pyrene (11) and dibenz[*ac*]anthracene (12) were Aldrich products. Numbers identify the compound in tables and figures.

Procedure

The appropriate weight of SDS was dissolved in Milli-Q (Millipore) water or in water with the desired alcohol content, and the solution filtered through

a 0.45μm nylon membrane filter (Whatman) to remove particulate matter. The mobile phase was degassed in an ultrasonic bath prior to use. Stock solutions of PAHs were prepared in ethanol and diluted with the mobile phase to obtain the desired concentration. These solutions were stored in the dark at 0°C to avoid possible degradation of PAHs.

The column temperature was controlled by immersion in a circulating water bath. Temperature was varied in the range 30°C to 60°C and adjusted to ±0.1°C. All temperature studies were performed in sequence from high to low temperature, according to other workers[17, 18] as this procedure eliminates solvent entrapment in the stationary phase. For chromatographic measurements the column was equilibrated to the desired temperature with at least 100 column volumes of micellar mobile phase until the detector signal was constant. Additionally, the mobile phase reservoir was thermostated during the experiments in order to obtain proper and quick temperature equilibration, and the injection volume was of 20μL.

Chromatograms shown were obtained with different solutions obtained by dilution of a stock solution of the following composition ($\mu g.mL^{-1}$): naphthalene (0.256), acenaphthylene (0.308), fluorene (0.332), anthracene (0.356), phenanthrene (0.356), 9-methylanthracene (0.384), fluoranthene (0.404), pyrene (0.405), chrysene (0.454), benzo[*b*]fluoranthene (0.126), benzo[*a*]pyrene (0.505), dibenz[*ac*]anthracene (0.139).

RESULTS AND DISCUSSION

The most serious problem described in the published reports on micellar liquid chromatography is the loss of efficiency when compared to traditional hydroorganic mobile phases. The plots of the height equivalent to theoretical plate (H) versus reduced velocity are very useful to compare efficiencies of chromatographic systems. From the chromatogram obtained the theoretical plate number (N) can be calculated for each peak according to the following equation 1

$$N = 5.54 \left(\frac{t_r}{w_{1/2}} \right)^2 \qquad (1)$$

where t_r is retention time and $w_{1/2}$ is the peak width at half peak height. However, this equation is highly inaccurate for skewed peaks.[19]

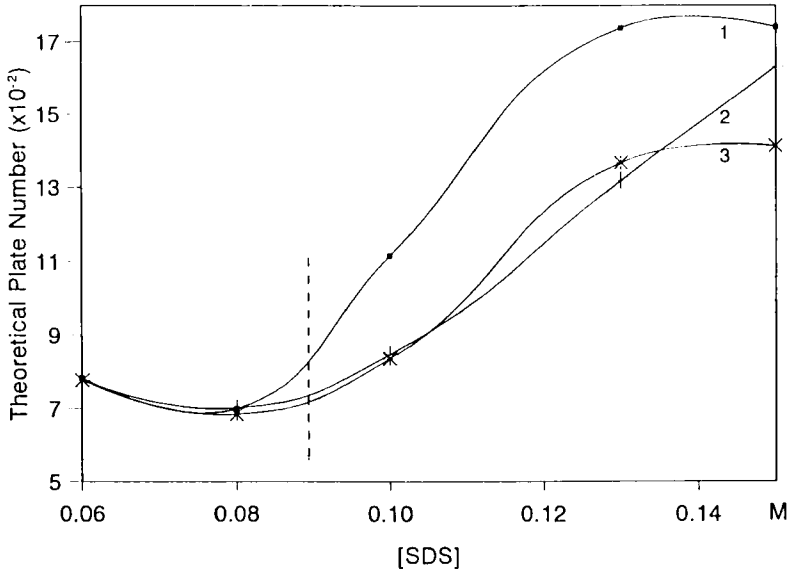

Figure 1. Variation of efficiency as a function of the concentration of SDS in the mobile phase for: 1) fluorene, 2) chrysene and 3) dibenz[*ac*]anthracene.

To determine column efficiency using information regarding peak asymmetry, the equation described by Foley and Dorsey[20] gives a more accurate measurement of column efficiency as the accuracy and precision values determined were a factor of two better than those found by Barber and Carr[21] equation. Thus Equation 2 [20]

$$N = \frac{41.7(t_r / w_{1/2})^2}{A/B + 1.25} \qquad (2)$$

was used in this work, where A/B is the asymmetry ratio measured at 10% peak height. The height equivalent to theoretical plate (H) can be calculated from the ratio H=L/N.

Effect of Concentration of Surfactant

Figure 1 shows the plot of the theoretical plate number (N) vs SDS concentration for three selected PAHs of different hydrophobicity. One can

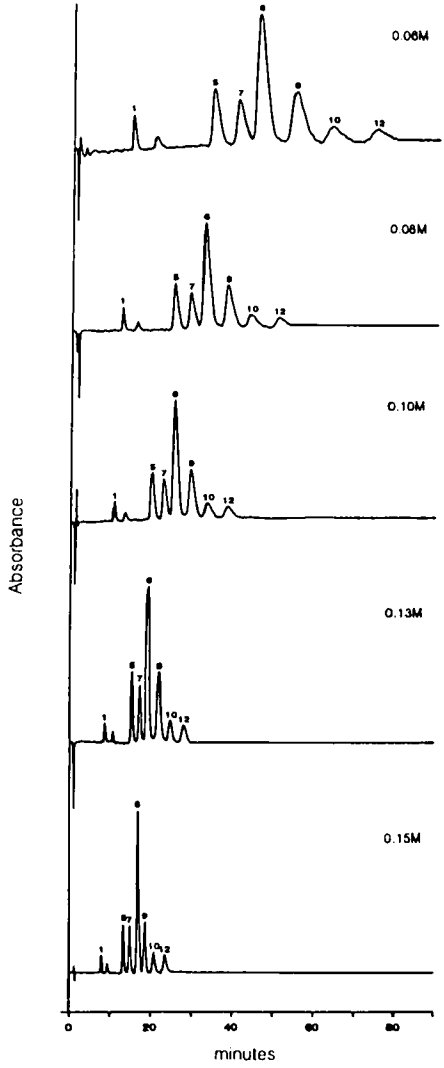

Figure 2. Chromatograms of a mixture of PAHs with different micellar mobile phases containing XM SDS + 15% 2-propanol. Flow rate 1.0 mL min^{-1} and column temperature 60°C.

observe that N increases drastically when increasing the concentration of the surfactant in the micellar mobile phase. This increment of efficiency is more pronounced for the less hydrophobic solute fluorene than for the more hydrophobic chrysene and dibenz[*ac*]anthracene. The degree of retention was large enough to keep the influence of extracolumn band broadening at an insignificant level, since k' values were greater than 8 for any solute at all concentrations. Figure 2 presents typical chromatograms for a mixture of seven PAHs to show the influence of the concentration of SDS on the efficiency of separation. One can observe that better separations can be obtained with high concentrations of SDS, showing a considerable change in the efficiency up to 0.13M SDS. From 0.13M to 0.15M SDS the variation in the effciency is minimum and only a slight improvement in the analysis time exists.

Effect of Type of Alcohol

The addition of small amounts of alcohols to MLC systems has been shown to improve column efficiencies significantly, especially when measured with hydrophobic analytes.[11,12] The most common reasoning is that the addition of alcohols reduces the loading of surfactant in the stationary phase which leads to improvements in the mass transfer and wetting of the stationary phase.[13] The concentration of alcohol, however, must not be very high because it might reduce the role of micelles and bring the system closer to a hydroorganic system. Notwithstanding, it has been described that percentages of 2-propanol up to ≈20% mantain the integrity of micelles, although in this case mixed micelles are formed.[22,23]

Table 1 shows plate counts and asymmetries for fluorene, chrysene and dibenz[*ac*]anthracene in a 0.15M SDS mobile phase, with 3% alcohol modifier. An improvement in both peak symmetry and efficiency can be observed from methanol to 2-propanol for the less hydrophobic fluorene and chrysene, while for the most hydrophobic dibenz[*ac*]anthracene the best symmetry is obtained using butanol as the modifier, even though the variations in efficiency are smaller. Thus, the value of efficiency is directly related to hydrophobicity of solutes. From Table I, one can deduce that higher efficiency will be obtained with an alcohol of intermediate chain such as 2-propanol.

Figure 3 shows an increase in efficiency when increasing the percentage of 2-propanol in the mobile phase up to 15%, from which an almost constant plate count is seen, and only a reduction in the analysis time exists.

To illustrate the efficiencies attainable with micellar mobile phases a

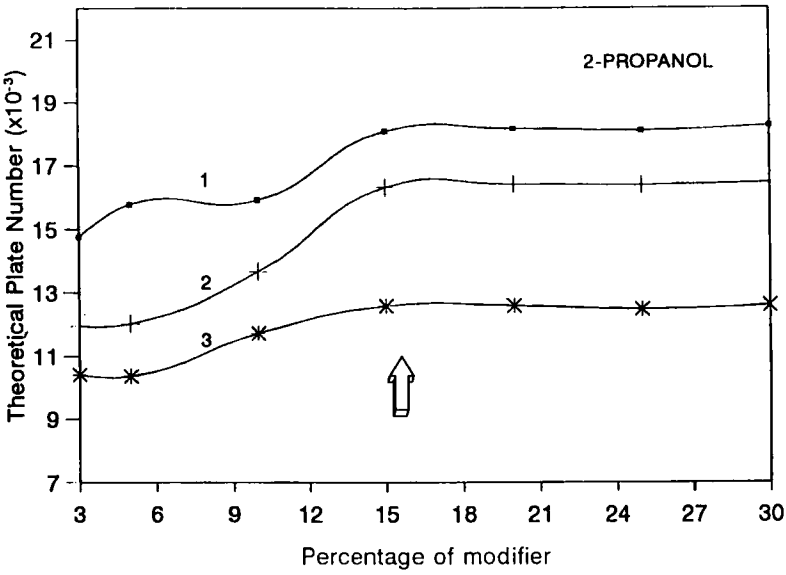

Figure 3. Variation of efficiency as a function of the percentage of 2-propanol in the mobile phases for: 1) fluorene, 2) chrysene and 3) dibenz[*ac*]anthracene.

Table 1

Variation of Efficiency and Asymmetry with Organic Modifiera

Solute	Organic Modifier					
	Methanol		2-Propanol		Butanol	
	N	A/B	N	A/B	N	A/B
Fluorene	890	1.36	1112	1.09	552	1.12
Chrysene	940	1.12	1195	1.03	644	1.32
Dibenz[ac]-anthracene	1133	1.66	1209	1.60	1092	1.17

Mobile phase 0.15M SDS with 3% of organic modifier, 30°C

series of chromatograms were run. Figure 4 is the separation of a mixture of seven PAHs using a 0.15M SDS mobile phase with variable percentage of 2-propanol. The separation efficiency improves substantially as the percentage

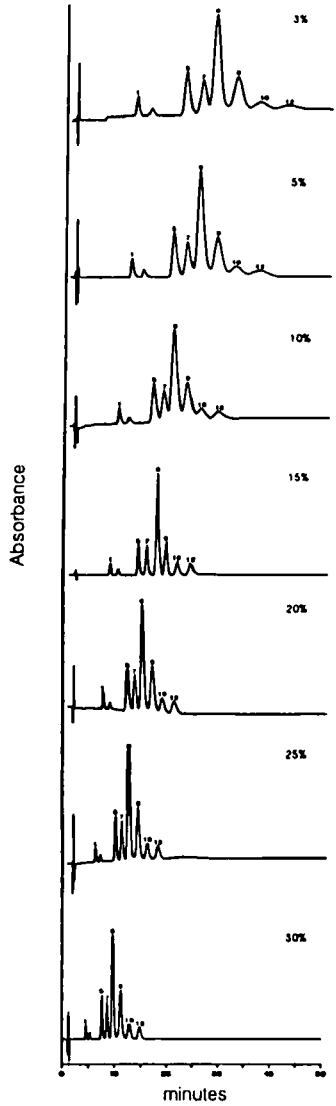

Figure 4. Chromatograms of a mixture of PAHs with a mobile phase of 0.15M SDS and variable percentages of 2-propanol. Flow rate 1.0 mL min^{-1} and column temperature 60°C.

of 2-propanol in the mobile phase increases, with a maximum at 15% 2-propanol. From this point up only slight improvements are obtained, while analysis time is reduced.

Effect of Temperature

An important parameter which can be adjusted to improve mass transfer characteristics is the temperature, because increasing temperature diminishes the viscosity of micellar mobile phases and improves mass transfer. We chose to investigate the influence of temperature on column efficiency across a wide range of eluent flow rates. The experimental data are used to construct H-μ plots, where H is the height equivalent to a theoretical plate and μ is the eluent linear velocity determined as the column length divided by the void time. Since the proper method for determination of the void time is not correct enough, particularly when a range of temperature is to be used, the linear velocity has been substituted for the flow rate ($mL.min^{-1}$).

Figure 5 shows plots of plate height versus flow rate for PAHs of different molecular weight at different temperatures. Contrary to what occurs in RP-HPLC, where an increase in column temperature leads to higher H values regardless of eluent flow rate, MLC is characterized by a general diminution of H as temperature increases. However, a different behavior exists as a function of the flow rate depending on the type of solute. For the lower molecular weight acenaphthylene and fluorene, Figures 2a and 2b show that H increases as flow rate increases up to 0.5 $mL.min^{-1}$, with a reversal order in the magnitude of H as temperature goes from 50 to 60°C. Greater increases in H from 1 $mL.min^{-1}$ on are observed at lower temperatures for these compounds. For higher molecular weight compounds such as anthracene and chrysene, Figs. 5c and 5d, this increase in plate height at lower temperatures is even sharper, and no reversal in H values exists at higher temperatures.

Table 2 shows the variation in asymmetry and plate counts for several PAHs using a 0.15M SDS+15% 2-propanol as mobile phase, as temperature is changed from 30 to 60°C. While the efficiency increases slightly, an improvement of peak shape is seen at higher temperatures.

Figure 6 illustrates the separation of nine PAHs using a mobile phase of 0.15M SDS + 15% 2-propanol at a constant temperature of 60°C. It is possible to see that efficiency increase from a flow rate of 0.5 $mL.min^{-1}$ to 1.0$mL.min^{-1}$, and that the resolution of separation as well as the analysis time is satisfactory. At flow rates higher than 1.5$mL\ min^{-1}$ the resolution is lost for the less hydrophobic solutes, being even much worse at the highest flow rates.

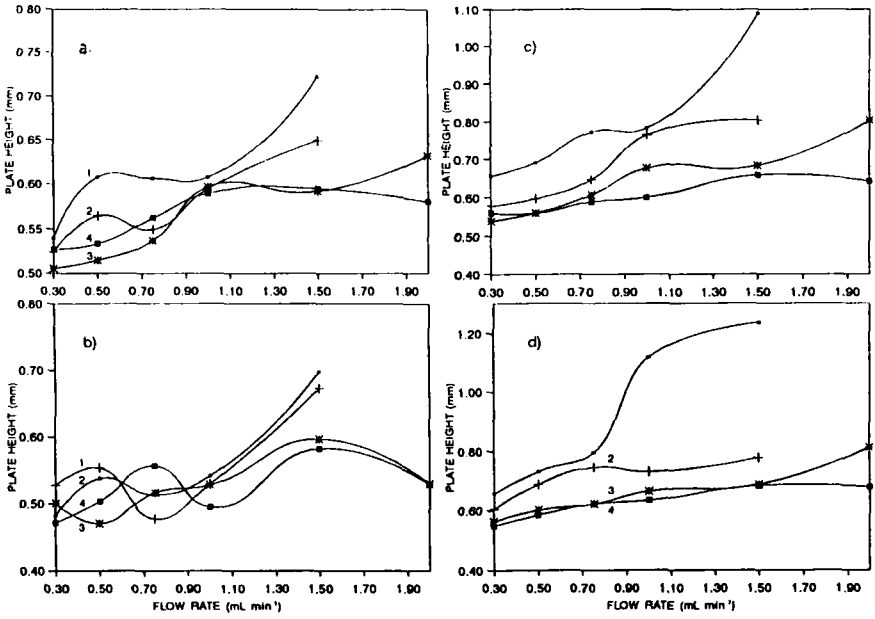

Figure 5. Experimental H,μ curves for: a) acenaphthylene, b) fluorene, c) anthracene, d) chrysene at different temperatures: 1) 30°C, 2) 40°C, 3) 50°C and 4) 60°C.

Table 2

Variation of Efficiency andAsymmetry with Temperature*

Solute	Temperature (°C)							
	30		40		50		60	
	N	A/B	N	A/B	N	A/B	N	A/B
Fluorene	1846	1.03	1889	1.02	1893	1.04	2021	1.03
Chrysene	1408	1.24	1367	1.12	1503	1.19	1577	1.17
Dibenz[ac]-anthracene	1290	1.20	1355	1.18	1281	1.19	1411	1.02

*Mobile phase 0.15M SDS with 15% of 2-propanol

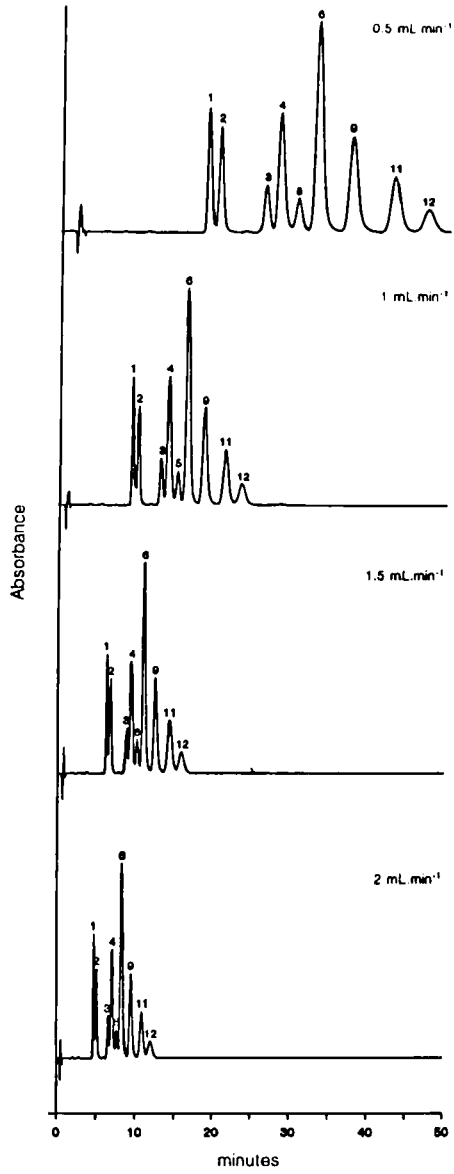

Figure 6. Chromatograms of a mixture of PAHs at different flow rates. Column temperature was 60°C and all mobile phases were modified with 15% 2-propanol.

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