EVALUATION OF THE USE OF ANIONIC/ NONIONIC MIXED MICELLES IN REVERSED PHASE LIQUID CHROMATOGRAPHY OF CHLOROPHENOLS

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Abstract

The use of aqueous mixed micellar system consisting of sodium dodecyl sulfate (SDS) and polyoxyethylene (23) dodecanol (Brij-35) as the mobile phase in reversed-phase liquid chromatography was studied. A group of chlorophenols was used as the test mixture. Adding organic modifier to the system showed that the use of low concentrations of organic additives improves efficiency in SDS/Brij-35 mixed micellar mobile phase. The effects of propanol concentration, total surfactant concentration and pH upon retention and selectivity were also assessed. It was shown that a linear correlation exists between LnK′ and volume fraction of propanol over a range of 0-10%. Also, a regular decrease in solutes retention was observed as a function of surfactant concentration. The results showed that the elution strength increases as the mobile phase pH decreases. It was also revealed that the chromatographic selectivity changes with an increase in elution strength.

Introduction

In the last two decades, the popularity of micellar liquid chromatography (MLC) as an alternative to reversed-phase liquid chromatography (RPLC) has increased. Micellar liquid chromatography has extended the capability of RPLC by allowing rapid gradient elution, detection sensitivity enhancement, unique separation selectivity, etc. [1-6]. The existence of solute-micelle interactions is a noticeable characteristic of micellar liquid chromatographic separations. In

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MLC, a solute can associate with the micellar pseudo phase through a combination of electrostatic, hydrophobic and steric interactions [7]. The type of interaction depends upon the solute and micelle properties. Thus, retention and selectivity can be more easily controlled by variation in type and concentration of surfactants.

Three important aspects of MLC including efficiency, solvent strength and selectivity in MLC using simple micelles have been extensively studied [7-10]. However, chromatographic characteristics of RPLC using micelles composed of mixture of surfactants and different structures seem to have been largely neglected. The major objective of this study was to investigate the feasibility and characteristics of using nonionic/anionic mixed micellar mobile phase in RPLC. This group of

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mixed micelles shows negative deviation from ideality such that the critical micelle concentration of the mixture of surfactants is lower than what is predicted based on the ideal solution theory [11].

In this paper, the results of a study on the effect of adding organic modifiers to the system on chromatographic efficiency are reported. The influence of organic modifier, micelle concentration and pH on elution strength and selectivity in mixed micellar liquid chromatography of chlorophenols is also described.

Experimental Section

Apparatus

The HPLC system consisted of a 6000A pump, M600 solvent programmer, and UK injector, all from Waters (Waters. Assoc. Milford, MA, USA) and a Perkin-Elmer (Norwalk, CT, USA) LC-95 UV detector set at 254 nm. The columns used were: 5 µ Waters Resolve C18 (150×3.9 mm I.D) and 10 µ Waters Bonda Pak C18 (300×3.9 mm I.D). Columns were thermostated at 40°C by a water circulator bath. The pH of mobile phase was measured with Ciba-Corning Model 250 pH meter.

Materials

The surfactants, SDS and Brij-35, and test solutes were used as received from Fluka. HPLC grade methanol (MeOH), ethanol (EtOH), propanol (PrOH), isopropanol (iso-PrOH) and butanol (BuOH) were also obtained from Fluka. The test solutes were 2-(2CP), 3-(3CP) and 4-chlorophenol (4CP), 2,3-(23CP), 2,4-(24CP), 2,5-(25CP) and 3,4-dichlorophenol (34CP), 2,4,5-(245CP), 2,4,6-(246CP) 2,3,6-trichlorophenol (236CP) and pentachlorophenol (pCP). The micellar solutions were prepared in double distilled, deionized water and were filtered through a 0.45 µm Millipore solvent filter. The stock solutions of chlorophenols (0.5-1 mg/ml) were prepared in methanol. The mobile phase pH was adjusted by adding phosphate buffer.

Methods

The chromatographic efficiency was calculated using the manual procedure of Foley and Dorsey [12], as Equation 1:

$$N = \frac{41.7(t_r/W_{0.1})^2}{(B/A)+1.25} \quad (1)$$

where t_r is the solute retention time, $W_{0.1}$ is the peak width measured at 10% peak height, and B/A is the peak asymmetry factor. The capacity factor, K′, was calculated in the usual manner using methanol as void marker.

Results and Discussion

Organic Modifier Effects on Chromatographic Efficiency

A major disadvantage of MLC is lower efficiency when compared to conventional reversed-phase liquid chromatography [7]. This low efficiency is caused by slow mass transfer due to poor wetting of the stationary phase. It has been shown that the efficiency can be improved by adding small quality of an organic modifier to the mobile phases containing simple micelles [8].

The effects of various alcohol additives on chromatographic efficiency using a SDS/Brij-35 mixed micellar eluent are presented in Table 1. Like other micellar mobile phases, an improvement is seen in both peak symmetry and plate count as the polarity of the organic modifier is decreased and wetting of the stationary phase is enhanced. Adding of low concentrations of organic modifiers also increases the elution strength and decreases the test solute retention. The results show that capacity factor decreases as the hydrophobicity of the organic co-solvent is increased.

The effect of the propanol concentration on chromatographic efficiency in a SDS/Brij-35 micellar mobile phase was also determined. As shown in Table 2, efficiency improves as a function of propanol concentration over a range of 0-5%. Little improvement is seen when concentration of propanol exceeds 5%.

Effect of Propanol Concentration on Retention

In hybrid systems (simple micelles in hydro-organic mobile phases), an increase in organic solvent concentration, causes an increase in the elution strength and decrease in the solutes retentions. The correlation between capacity factor and volume fraction of organic modifier (φ) can be written as Equation 2 [13]:

$$\mathrm{Ln}K' = -S_{hyb}\varphi + \mathrm{Ln}K'_o \quad (2)$$

where S_{hyb} is solvent strength parameter in hybrid system and K'_o is the capacity factor in purely aqueous micellar eluent.

Equation 2 adequately describes (r>0.98) the retention behavior of the test mixture of chlorophenols in SDS/Brij-35 mixed micellar LC as a function of volume fraction of propanol over a range of 0-10% (Table 3). The degree of reduction in retention isn't the same for different solutes and this can lead to changes in selectivity. The correlation between S and $\mathrm{Ln}K'_o$ for chlorophenols is illustrated in Figure 1. As shown, the S parameter is often inversely related to retention. In MLC using simple micelles, this implies a selectivity enhancement as a result of an increase in volume fraction of organic solvent. The influence of propanol concentration on selectivity for SDS/Brij-35 micellar system is shown in Figure 2. As can be seen, in presence of SDS/Brij-35 mixed micelles, selectivity variations don't occur systematically with volume

fraction of propanol added to micellar eluents.

Table 1. Effect of organic modifiers on chromatographic efficiency

Organic modifier	K′	N	B/A
None	12.75	2775	1.40
MeOH	12.06	3212	1.27
EtOH	11.54	3525	1.18
i-PrOH	11.00	3729	1.05
PrOH	10.00	3861	1.05
BuOH	6.75	4206	1.05

Conditions: 150 mm 5 µ C18 column, flow rate 0.9 ml/min, mobile phase 25 mM SDS+25 mM Brij-35+5%(v/v) organic modifier, 40°C, 2CP test solute.

Table 2. Effect of Propanol concentration on chromatographic efficiency

Propanol (v/v%)	0.0	1.0	3.0	5.0	7.0	10.0
N	2775	3096	3504	3861	3038	2988
B/A	1.40	1.15	1.05	1.05	1.07	1.20

Conditions are as in Table 1.

Table 3. Results of regression analysis from LnK′ *vs* volume fraction of Propanol

Solute	Slope	Intercept	r
2CP	-5.537E-2	2.522	-0.9807
4CP	-5.430E-2	2.584	-0.9827
3CP	-5.209E-2	2.640	-0.9819
23CP	-4.954E-2	2.757	-0.9800
34CP	-5.026E-2	2.581	-0.9842
24CP	-4.928E-2	2.838	-0.9849
25CP	-4.427E-2	2.997	-0.9874
236CP	-4.845E-2	2.990	-0.9800
246CP	-4.587E-2	3.033	-0.9933
245CP	-5.895E-2	3.154	-0.9898
pCP	-4.719E-2	3.300	-0.9827

Conditions: 150 mm 5 µ C18 column, flow rate 0.9 m1/min, mobile phase 25 mM SDS +25 mM Brij-35 and 0-10% propanol, 40°C.

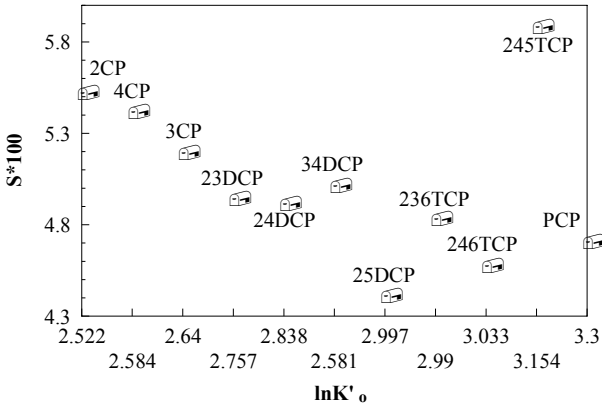

Figure 1. Relation between S_{hyb} value and retention in SDS/ Brij-35 micellar mobile phase. Conditions are as in Table 3.

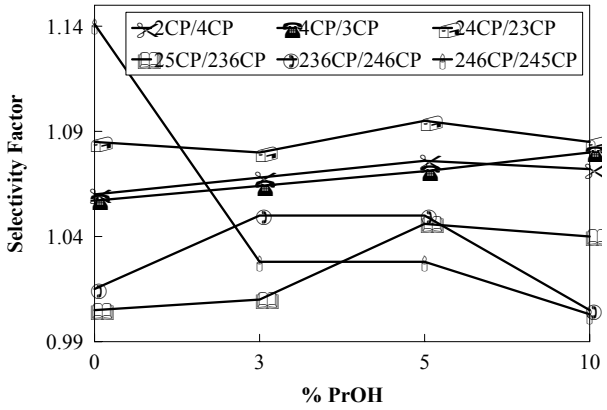

Figure 2. Variation of selectivity with the volume fraction of PrOH in SDS/Brij-35 micellar mobile phase. Conditions are as in Table 2.

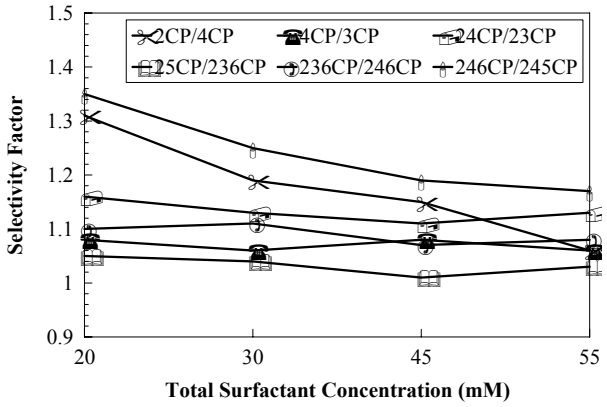

Figure 3. Influence of total surfactant concentration on selectivity in SDS/Brij-35 micellar mobile phase at Brij-35 mole fraction of 0.3 using 300 mm 5 µ C18 column, flow rate 1.2 ml/min and 3% PrOH, 40°C.

Effects of Surfactant Concentration on Retention and Selectivity

The effect of simple micelles on retention in RPLC has been reported by a number of authors [5,14,15]. They showed that an increase in surfactant concentration usually leads to a decrease in retention.

In MLC using SDS/Brij-35 mixed micellar eluent, a good correlation (r>0.99) between 1/K′ and total surfactant concentration was observed for all solutes (Table 4). Our results show that the solutes studied in this work are highly bonding solutes with SDS/Brij-35 mixed micellar mobile phase. The degree of decrease in retention for different compounds varies depending on their partition coefficients into mixed micelles and stationary phase. Due to different types of the competing equilibria in MLC, one can expect any form of selectivity behavior as a result of a change in micelle concentration. Figure 3 shows the variation of selectivity with the total surfactant concentration in micellar mobile phase. As shown, convergence, divergence or reversal of the peaks can occur as a result of an increase in total surfactant concentration.

Mobile Phase pH Effects

The pH of the micellar eluent is an important factor for the analysis of ionizable compounds. The influence of the mobile phase pH on the chromatographic behavior of chlorophenol compounds was studied. The results are presented in Table 5. The capacity factor is found to increase as the mobile phase pH is increased. Retention in MLC is inversely related to the solute-micelle binding constant (K_m) in following form [12]:

$$K' = (P_s \Phi)/(K_m[M]+1) \quad (3)$$

where P_s is the partition coefficient of solute between the mobile and stationary phases, Φ is the phase ratio and [M] is the micelle concentration. Therefore, it is concluded that K_m for all solutes decreases with an increase in the mobile phase pH. This behavior can be attributed to the fact that the activation barriers to sorption/desorption is greater in SDS/Brij-35 systems at higher pH due to greater electrostatic hinderence to mass transfer. The change in the mobile phase pH affects nonionic Brij-35 surfactants because the ether linkage in those polyoxyethylene chains can be protonated at low pH yielding positively charged groups [16]. Based on this assumption, one can be expected that protonated nonionic surfactants reduce the net electrical charge density of anionic surfactants in SDS/Brij-35 mixed micellar systems. Our observation also indicates that a decrease in pH usually leads to a decrease in selectivity (Fig. 4). The chromatograms of chlorophenols for two different SDS/Brij-35 micellar mobile phases are illustrated in Figures 5 and 6.

Table 4. Results of regression analysis from 1/K′ *vs* total surfactant concentration

Solute	Slope	Intercept	r
2CP	9.828E-4	1.240E-2	0.9940
4CP	1.034E-3	4.207E-3	0.9999
3CP	9.896E-4	3.138E-3	0.9995
23CP	9.414E-4	-3.052E-3	0.9998
34CP	8.483E-4	-2.810E-3	0.9997
24CP	8.448E-4	-2.931E-3	0.9998
25CP	7.172E-4	6.034E-4	0.9999
236CP	7.069E-4	1.741E-3	0.9994
246CP	6.828E-4	3.966E-4	0.9999
245CP	6.483E-4	-2.810E-3	0.9995
pCP	5.552E-4	9.310E-4	0.9971

Conditions: 300 mm 5 µ C18 column, flow rate 1.2 m1/min, SDS/Brij-35 micellar mobile phase at Brij-35 mole fraction of 0.3 and 3% propanol, 40°C.

Table 5. Capacity factor values of chlorophenols as a function of pH

pH		2.8	4.7	6.4
No.	Solute		K′	
1	2CP	8.17	8.58	9.63
2	4CP	8.67	9.17	10.42
3	3CP	9.23	9.83	11.15
4	23CP	10.67	11.33	12.50
5	34CP	11.25	12.00	13.33
6	24CP	11.17	12.33	13.50
7	25CP	12.67	14.00	15.17
8	236CP	14.60	14.83	15.75
9	246CP	14.83	15.17	16.58
10	245CP	15.16	16.00	17.67
11	pCP	18.50	19.00	19.33

Conditions: 150 mm 5 µ C18 column, mobile phase 25 mM SDS+25 mM Brij-35+3% propanol and flow rate 0.7 m1/min. The pH of mobile phase was adjusted with phosphate buffer, 40°C.

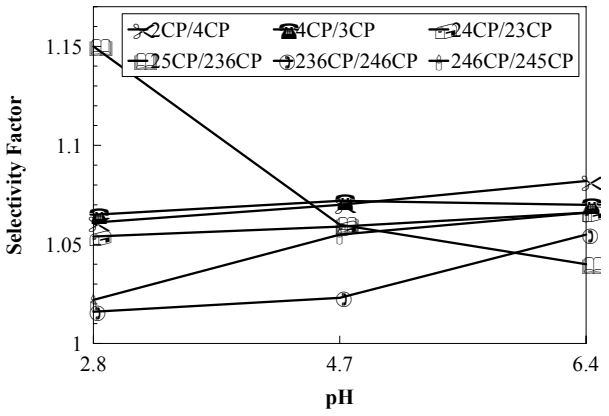

Figure 4. Variation of selectivity with pH in SDS/Brij-35 micellar mobile phase. Conditions are as in Table5.

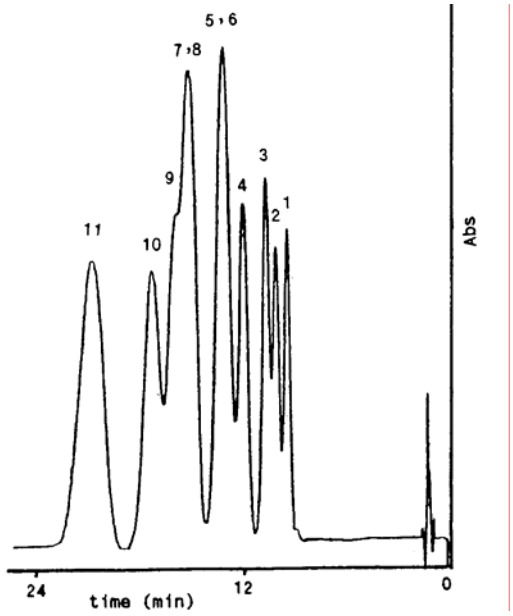

Figure 5. The measured chromatogram for chlorophenols defined in Table 5 using 150 mm 5 µ C18 column, 50 mM SDS/Brij-35 micellar mobile phase at Brij-35 mole fraction of 0.5, 5% PrOH and flow rate 0.9 ml/min, 40°C.

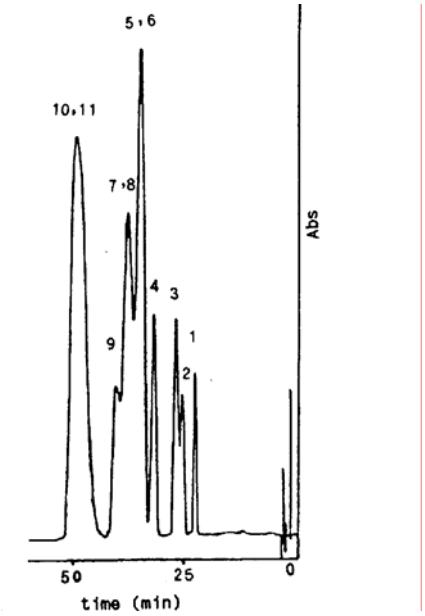

Figure 6. The measured chromatogram for chlorophenols using 300 mm 5 µ C18 column, 45 mM SDS/Brij-35 micellar mobile phase at Brij-35 mole fraction of 0.3, 3% PrOH and flow rate 1.2 ml/min, 40°C. The identification numbers of the solutes refer to Table 5.

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