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Optimization of the separation selectivity of a group of benzene and naphthalene derivatives in micellar high-performance liquid chromatography using a C_{18} column and alcohols as modifiers in the mobile phase

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ABSTRACT

The separation selectivity of fifteen benzene and naphthalene derivatives in micellar high-performance liquid chromatography, using a C_{18} column, was studied as a function of the parameters on which it depends. A multiple linear regression programme was used to find the dependence of the selectivity coefficient on the following parameters: nature of the surfactant in the mobile phase (sodium dodecyl sulphate or hexadecyltrimethylammonium bromide), surfactant concentration (0.02–0.1 *M*), nature of the additive in the mobile phase (methanol, *n*-propanol, *n*-butanol and sodium chloride) and percentage of the alcohol (0, 5 or 10%). Selectivity optimization corresponds to the use of sodium dodecyl sulphate at low concentrations and the addition of an alcohol of medium chain length.

INTRODUCTION

The use of surfactant solutions, at a concentration above the critical micelle concentration (c.m.c.) as mobile phases for reversed-phase liquid chromatography has received much attention. The popularity of micellar liquid chromatography (MLC) is due to its ability for the simultaneous separation of ionic and non-ionic compounds, rapid gradient elution, possibility of direct injection of physiological fluids, enhancement of fluorescence and absorption detection, etc. [1–11].

The interaction between solutes and micelles can be evaluated through the calculation of the solute–micelle binding constant using equations that describe solute retention as a function of micelle concentration, based on a three-way partition model proposed by Armstrong and Nome [4] and Arunyanart and Cline-Love [5]. These equations have also been experimentally verified for a large number of organic solutes [12–20]. The reported values for the solute–micelle binding constant can be used to facilitate systematic optimization in MLC [14].

As in reversed-phase liquid chromatography (RPLC), selectivity in MLC is primarily controlled by the composition of the mobile phase, *i.e.*, the type and concentration of surfactant present as micellar aggregates. The solute behaviour in MLC was attributed to different factors such as the special association of solutes with micelles through a combination of electrostatic, hydrophobic and steric interactions [21,22], the heterogeneous nature of micelles

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that provides a different microenvironmental polarity for compounds in a given mobile phase, and the existence of two competing equilibria, namely solute partitioning in the stationary phase and solute partitioning in the mobile phase micelles [6,15,23,24].

A serious drawback of all MLC systems studied to date is their poor chromatographic efficiency. This deficiency is most important when viewed in the context of resolution when compared with that of commonly used aqueous organic mobile phases [25]. Poor wetting of the stationary phase [26] and restricted mass transfer [27] are the reasons for the decrease in efficiency. Several workers have investigated this aspect. Dorsey *et al.* [26] proposed the use of organic modifiers, addition of 3% of *n*-propanol to the micellar mobile phase and an elevated column temperature. Yarmchuck *et al.* [27] recommended the use of low mobile phase flow-rates, elevated operating temperatures and minimum surfactant concentrations. It was also suspected that surfactant adsorption on the stationary phase had a great impact on the MLC efficiency [28–31]. It was shown that the addition of a short- or medium-chain alcohol causes surfactant desorption from the stationary phase and improves the efficiency [32].

Surprisingly, the effect of organic modifiers on chromatographic selectivity has been ignored for a long time and only a few papers have appeared. Khaledi and co-workers [15,23] studied the effect of organic solvents on retention and methylene group selectivity in MLC, and the effect of adding organic solvents to micellar eluents on the chromatographic selectivity of polar and ionic solutes [24,33,34]. More recently, they studied the role of organic modifiers and micelles in controlling solvent strength and selectivity in MLC [35,36].

Achieving a satisfactory separation within a reasonable run time requires the selection of experimental conditions that optimize the separation factor (α), the column plate number (N) and the solute capacity factor (k') [37]. Our aim in this work was the study of MLC selectivity in terms of the separation factor. The effect of the nature and concentration of the surfactant and the type and concentration of the additive used in the mobile phase (methanol, *n*-propanol, *n*-butanol and sodium chloride) on separation selectivity was studied. Chemometric methods were also applied to the retention data for fifteen aromatic compounds (benzene and naphthalene derivatives) to find the optimum analytical conditions.

EXPERIMENTAL

Apparatus

The chromatograph consisted of a Model 510 pump, a Model U6K injector, a Model 440 fixed-wavelength (254 nm) detector and a Model 740 data module (all from Waters). Retention data were obtained with a 15 cm × 3.9 mm I.D. Spherisorb ODS 2 ($d_p = 5$ μm) column (Teknokroma) and a 15 cm × 3.9 mm I.D. Nova-Pak C_{18} ($d_p = 4$ μm) column (Waters). Final separations were achieved on a 10 cm × 4.0 mm I.D. Hypersil ODS ($d_p = 3$ μm) column (Teknokroma). A 0.45-μm filter and filtration system (Millipore) were used. A Model 522 conductimeter (Crison) was employed.

Reagents

The surfactants sodium dodecyl sulphate (SDS) and hexadecyltrimethylammonium bromide (CTAB) (Merck), methanol (Scharlau) and *n*-propanol and *n*-butanol (Merck), were used as received.

Benzene and naphthalene derivatives of analytical-reagent grade were as follows: (1) benzene, (2) benzylic alcohol, (3) benzamide, (4) toluene, (5) benzonitrile, (6) nitrobenzene, (7) phenol, (8) 2-phenylethanol, (9) chlorobenzene, (10) phenylacetonitrile, (11) 3,5-dimethylphenol, (12) naphthalene, (13) 1-naphthol, (14) 2-naphthol and (15) 1-naphthylamine. Water purified with a Milli-Q system (Millipore) was used.

Procedure

Micellar mobile phases (with a surfactant concentration from 0.02 to 0.1 M) were prepared by dissolving the appropriate amount of surfactants and methanol, *n*-propanol or *n*-butanol in water in a ultrasonic bath followed by filtration. Stock solutions of test solutes were prepared in the

mobile phase itself and their concentrations were adjusted to permit their detection from the injection of a 20-μl volume of sample.

The void volume for SDS micelles was determined from the retention time of the peak originating from the injection of the nitrate anion into the chromatographic system. For CTAB mobile phases, the first deviation of the baseline was employed.

The column and the mobile phase were water jacketed and thermostated at 25 ± 1°C with a circulating water bath.

Determination of the c.m.c. for SDS–10% methanol solutions was achieved by conductivity measurements at constant temperature (25 ± 1°C).

TABLE I

CRITICAL MICELLAR CONCENTRATIONS OF MICELLAR SYSTEMS USED AS MOBILE PHASES

Micellar system	C.m.c. (M)	Ref.
SDS	$8.08 \cdot 10^{-3}$	38
SDS–10% MeOH	$8.20 \cdot 10^{-3}$	This work
SDS–10% PrOH	$4.70 \cdot 10^{-3}$	39
SDS–5% BuOH	$1.34 \cdot 10^{-3}$	40
SDS–10% BuOH	$2.27 \cdot 10^{-4}$	40
SDS–0.1 M NaCl	$1.40 \cdot 10^{-3}$	41
CTAB	$9.20 \cdot 10^{-4}$	38
CTAB–5% PrOH	$2.69 \cdot 10^{-3}$	42
CTAB–10% PrOH	$1.94 \cdot 10^{-3}$	42
CTAB–5% BuOH	$8.80 \cdot 10^{-4}$	43

RESULTS AND DISCUSSION

The capacity factors of fifteen benzene and naphthalene derivatives in an MLC system in the presence of methanol and n-propanol were determined by using SDS and CTAB as surfactants in the mobile phase. The results obtained were compared with those obtained previously for the same compounds in the absence of modifiers [12] and in the presence of n-butanol and sodium chloride [13]. All these data allowed conclusions to be drawn regarding the separation selectivity, the effects of the nature and concentration of the surfactant and the effects of the nature and percentage of alcohol used in the mobile phase. The c.m.c. values for SDS and CTAB in the absence and presence of the different alcohols are given in Table I.

Variation of the capacity factor

Figs. 1 and 2 show the variation of the logarithm of the capacity factor (log k') for the fifteen benzene and naphthalene derivatives as a function of the SDS and CTAB concentration, respectively. In both instances, the mobile phase was modified with 5% of n-butanol. For all compounds, the retention decreases when the eluent strength increases, as expected. The rate of change in retention of the different solutes varies with the solute charge and hydrophobicity and the length of the alkyl chain, charge and concentration of the micelles [35]. Regarding selectivity, in Figs. 1 and 2 and also in similar figures obtained with other mobile phases, it was observed that the separation selectivity increases when the surfactant concentration in mobile phase decreases for both surfactants (SDS and CTAB). An enhancement in selectivity as a result of decreasing micelle concentration has

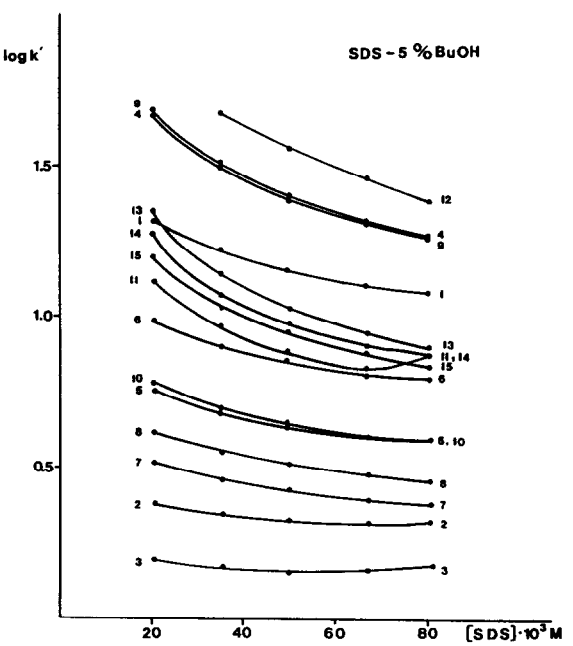

Fig. 1. Variation of log k' for the studied compounds (for numbers see *Reagents*) as a function of the concentration of SDS in a mobile phase modified with 5% butanol. Column: Nova-Pak C_{18} (15 cm × 3.9 mm I.D.) (d_p = 4 μm). Data from ref. 13.

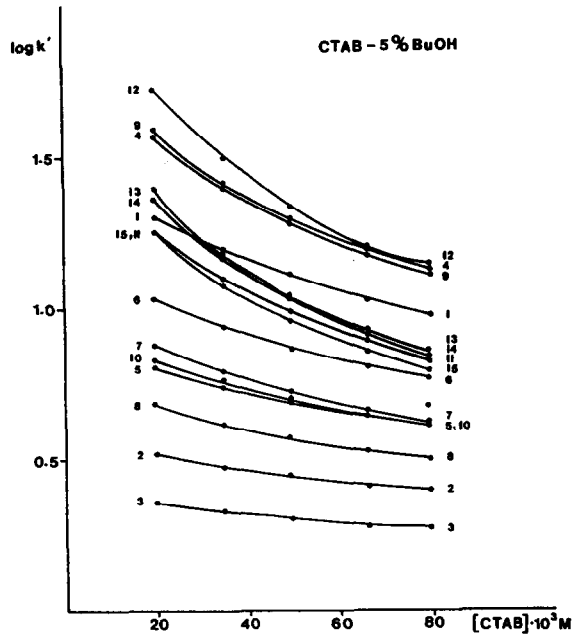

Fig. 2. Variation of log k' for the studied compounds as a function of the concentration of CTAB in a mobile phase modified with 5% butanol. Column: Nova-Pak C_{18} (15 cm × 3.9 mm I.D.) ($d_p = 4$ μm). Data from ref. 13.

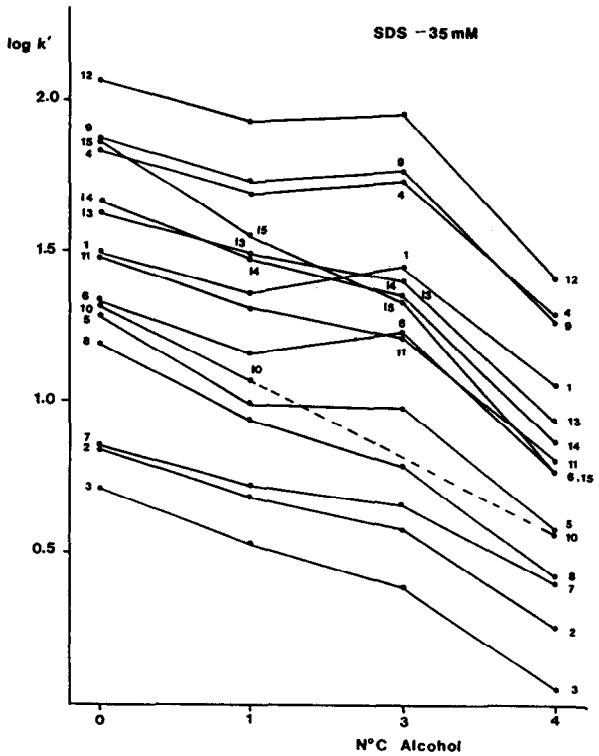

Fig. 3. Variation of log k' for the studied compounds as a function of the number of carbon atoms in the alcohol added at 10% to a mobile phase of 0.035 M SDS. Column: Nova-Pak C_{18} (15 cm × 3.9 mm I.D.) (d_p = 4 μm), except Spherisorb ODS-2 (15 cm × 3.9 mm I.D.) ($d_p = 5$ μm) for n-propanol. Data for aqueous mobile phases from ref. 12 and data for n-butanol from ref. 13.

also been observed by other workers [35]. On the other hand, in all the mobile phases for which the comparison between SDS and CTAB was possible, the separation selectivity was, in general, better for SDS than for CTAB. Taking into account that CTAB eluents are inherently stronger for uncharged solutes owing to the longer surfactant chain length and that selectivity increases with decreasing micelle concentration, it seems that the separation selectivity decreases with increasing eluent strength (through an increase in concentration and chain length of the micelles).

In order to study the influence of the number of carbon atoms in the alcohol on the retention and selectivity for the fifteen compounds studied, the variation of log k' as a function of this parameter is plotted in Fig. 3 for a 0.035 M SDS mobile phase modified by a fixed percentage of each alcohol (10%). The results obtained in the absence of alcohol [12] are also included. Fig. 3 shows that the behaviour of all the compounds is similar. Retention, in general, decreases when the number of carbons in the alcohol increases. These results are in agreement with those obtained by other workers showing that butanol is the strongest and methanol the weakest solvent as in conventional aqueous–organic systems [24]. The larger solvent strength for butanol and propanol indicates that these solvents have a stronger interaction with micelles and, consequently, can solvate more effectively or can compete better with micelles for interaction with solutes. Six of the fifteen compounds (benzonitrile, nitrobenzene, benzene, toluene, chlorobenzene and naphthalene) show a retention with SDS–propanol eluent equivalent to or even larger than that with SDS–methanol. This result may be due to the fact that for n-propanol a different column was used, as indicated in Fig. 3.

Regarding selectivity, Fig. 3 shows that there are some pairs of compounds for which the separation selectivity is poor for any alcohol–chlorobenzene–toluene. On the other hand, the separation selectivity for some pairs decreases when the number of carbon atoms in the alcohol increases, which is the case with phenol–2-phenylethanol and 3,5-dimethylphenol–nitrobenzene. However, the separation selectivity increases for a greater number of pairs with increasing number of carbon atoms in the alcohol. It is possible, therefore, to state that even if the variation of the separation selectivity as a function of the nature of the alcohol depends on the nature of the compounds, in general terms *n*-butanol allows better selectivities than methanol or *n*-propanol to be obtained. These results indicate that separation selectivity in MLC increases with increasing solvent strength, in contrast to that obtained with conventional aqueous–organic systems where an increase in solvent strength causes a decrease in selectivity.

Fig. 4 shows the variation in log k' for the compounds studied as a function of the percentage of *n*-butanol in a 0.035 *M* SDS mobile phase. The eluent strength increases as the organic modifier concentration increases, resulting in a decrease in retention. In fact, it was observed that the retention for all compounds decreases in the presence of *n*-butanol, this reduction being more significant from 0% to 5% of alcohol than from 5% to 10%. The selectivity is better in the presence of *n*-butanol than in its absence, although there are some exceptions relating to the pairs which in Fig. 3 showed a decrease in selectivity with increasing number of carbons in the alcohol, *i.e.*, phenol–2-phenylethanol and 3,5-dimethylphenol–nitrobenzene. The fact that the separation selectivity is better in the presence of an alcohol agrees with the results obtained by other workers, who found in MLC a simultaneous enhancement of elution strength and selectivity [24,35]. However, Fig. 4 shows that the separation selectivity is slightly better at 5% *n*-butanol. This result can be justified by the existence of pairs of compounds for which the selectivity increases when the eluent strength decreases. A medium eluent strength can allow maximization of the separation selectivity for all kinds of compounds. A model has been developed [35] explaining the dependence of the solvation ability of organic solvents in MLC (represented by the solvent strength parameter, *S*, of solutes) and the degree of solute interactions with micelles. Whenever the difference in solvent strength parameter values of two solutes in micellar eluents, d*S*, is positive, maximum selectivity is observed at the weakest eluent strength. When the above-mentioned difference d*S* is negative, there exists an inverse relationship between the retention and solvent strength parameter so that the selectivity increases with increasing volume fraction of organic modifier in micellar eluents. The pairs whose separation selectivity decreases when the organic modifier concentration increases (phenol–2-phenylethanol and 3,5-dimethylphenol–nitrobenzene) are the same pairs whose selectivity decreases with increasing number of carbons in

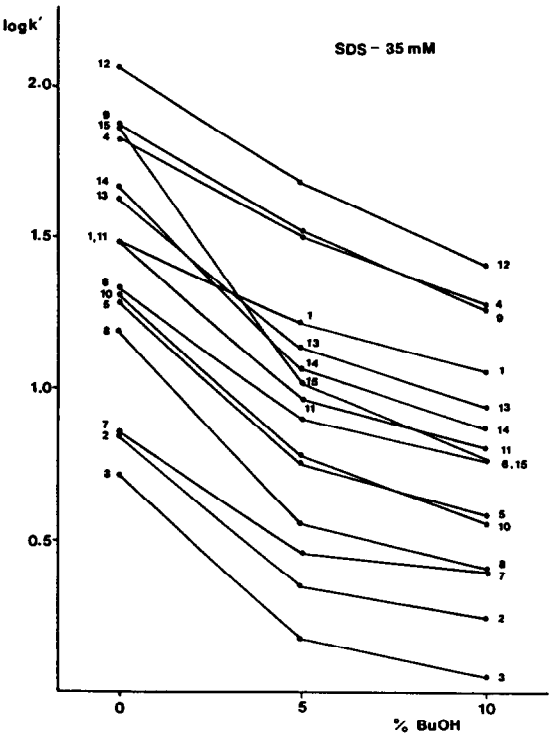

Fig. 4. Variation of log k' for the studied compounds as a function of the percentage of *n*-butanol in a mobile phase of 0.035 *M* SDS. Column: Nova-Pak C_{18} (15 cm × 3.9 mm I.D.) (d_p = 4 μm). Data from refs. 12 and 13.

the alcohol, that is, their selectivity also decreases when the eluent strength is increased by changing the chain length of the alcohol.

The variation of log k' as a function of the percentage of *n*-propanol in a 0.035 *M* CTAB mobile phase was measured to study the influence of the percentage of other alcohols. The results are shown in Fig. 5. It is observed that the effect that *n*-propanol has on the retention of the compounds in a CTAB mobile phase is similar to the effect that *n*-butanol has on the retention of the compounds in an SDS mobile phase. Retention decreases when the percentage of *n*-propanol in the mobile phase increases, that is, when the solvent strength also increases. In this instance maximum selectivity is obtained at 3% *n*-propanol, a percentage with which a greater number of pairs can be separated with a CTAB mobile phase. Again, a medium alcohol percentage seems to give better selectivity.

The addition of 0.1 *M* sodium chloride to an SDS mobile phase did not allow the selectivity of the separation to be increased significantly.

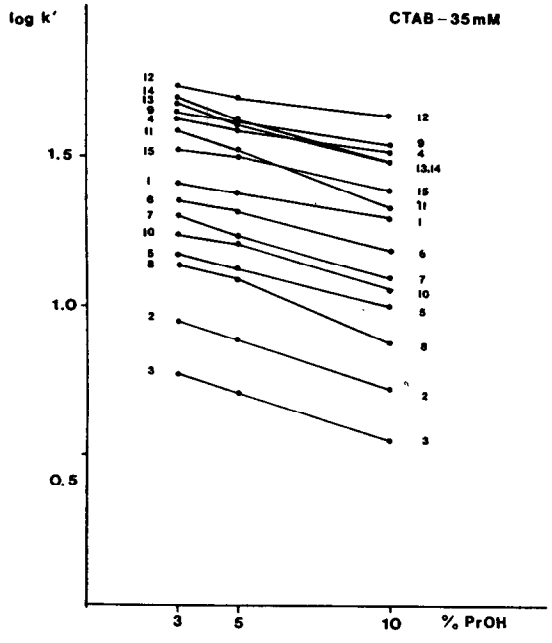

Fig. 5. Variation of log k' for the studied compounds as a function of the percentage of *n*-propanol in a mobile phase of 0.035 *M* CTAB. Column: Spherisorb ODS-2 (15 cm × 3.9 mm I.D.) ($d_p = 5$ μm).

Multiple regression study

To optimize the separation in MLC, a statistical study of the selectivity under the different experimental conditions studied was performed. The selectivity coefficient (α), defined as the ratio between the capacity factors of two compounds, was calculated for the pairs that can be obtained from the fifteen compounds under the different experimental conditions.

To study these results by means of a multiple linear regression programme, only selectivity coefficients that vary with the experimental conditions were chosen. This implies the exclusion of the selectivity coefficients of pairs that are either never separated or that can always be separated regardless of the experimental conditions. Therefore, the number of pairs considered in the programme was equal to 25 under the different experimental conditions which involved 45 different possibilities excluding 5 concentrations of SDS modified with 0.1 *M* NaCl.

The average value of the selectivity coefficient for the 25 pairs studied was calculated ($\bar{\alpha}$). The absolute value of $(\overline{1-\alpha})(|\overline{1-\alpha}|)$ is chosen as the dependent variable for using the programme. The greater is $|\overline{1-\alpha}|$, the better is the selectivity. The independent variables used in the programme and the possible values for each are given at Table II.

The best results correspond to the combina-

TABLE II

ASSIGNED VALUES FOR THE INDEPENDENT VARIABLES USED IN THE MULTIPLE REGRESSION ANALYSIS

Variable	Symbol	Assigned values
Nature of surfactant	*A*	1 (SDS) 2 (CTAB)
Surfactant concentration (mol/l)	*B*	0.020 0.035 0.050 0.067 0.080 0.100
Carbon number of the alcohol	*C*	0, 1, 3, 4
Alcohol percentage (v/v)	*D*	0, 5, 10

tion of the variables C and D, *i.e.*, assuming that the number of carbons in the alcohol and its percentage operate together.

The parameter $|\overline{1-\alpha}|$ can be expressed by the equation

$$|\overline{1-\alpha}| = 1.05(\pm 6.60\cdot 10^{-2}) - 0.22(\pm 2.97\cdot 10^{-2})A - 3.27\cdot 10^{-3}(\pm 6.28\cdot 10^{-4})B + 6.51\cdot 10^{-3}(\pm 1.16\cdot 10^{-3})CD \quad (1)$$

$n = 25$; $r^2 = 0.7784$; $s = 0.098$; $F = 48.01$

where the values in parentheses are the standard errors.

Although the signs and the magnitudes of the coefficients of a regression equation may not have any physical meaning, it is interesting to compare the information that could be obtained from these coefficients and from the experimental results. The negative coefficient obtained for the variables A and B could indicate an increase in the separation selectivity when the variables A and B decrease, *i.e.*, for the use of SDS as a surfactant and for low concentrations of surfactant in the mobile phase. This agrees with the experimental results presented here. The positive coefficient obtained on combining variables C and D implies that the selectivity increases when the variables C and D also increase, *i.e.*, the effect of the percentage of alcohol increases when the length of the chain of the alcohol also increases. Hence it is easier to modify the selectivity by means of the percentage of the alcohol when it has the maximum number of carbons (*n*-butanol). The experimental results show maximum selectivity for *n*-butanol at a level of 5%. Eqn. 1 provides the best combination for coefficient values that result in the least error in predicting $(\overline{1-\alpha})$.

Separation of mixtures

To test the validity of the above-mentioned conditions for the optimization of a separation, a mixture of the fifteen benzene and naphthalene derivatives was injected into an MLC system in which a C_{18} column ($d_p = 3$ μm) was used. The mobile phase chosen was to contain 0.035 *M* SDS. As modifiers *n*-propanol and *n*-butanol as alcohols with a greater number of carbons in the molecule and at concentrations of 5% and 10% were tested. Mobile phases containing 0.035 *M* SDS and 10% *n*-propanol or *n*-butanol allowed the separation of twelve peaks, but the pairs that could not be separated were not the same for the two mobile phases. With 10% *n*-butanol, phenol–2-phenylethanol, nitrobenzene–1-naphthylamine and toluene–chlorobenzene and with 10% *n*-propanol, benzonitrile–phenylacetonitrile, nitrobenzene–3,5-dimethylphenol and

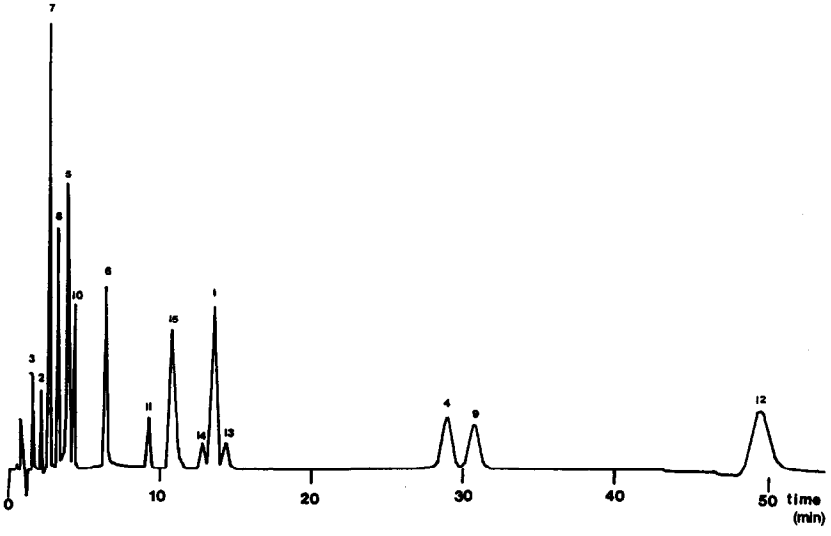

Fig. 6. Chromatogram for the separation of a mixture of fifteen benzene and naphthalene derivatives by using a 0.020 *M* SDS mobile phase modified with 5% butanol. Column: Hypersil ODS (10 cm × 4.0 mm I.D.) ($d_p = 3$ μm).

toluene–chlorobenzene could not be separated. It is important to emphasize that when the separation selectivity is similar for *n*-butanol and *n*-propanol, *n*-butanol provides a shorter analysis time. A concentration of 5% *n*-propanol or *n*-butanol allowed the separation of fourteen peaks from the mixture using a 0.035 *M* SDS mobile phase. The pair that could not be separated with 5% *n*-butanol was toluene–chlorobenzene and that with 5% *n*-propanol was benzonitrile–phenylacetonitrile. In this instance also (5% alcohol) the analysis time was shorter with *n*-butanol than *n*-propanol.

In order to separate all fifteen benzene and naphthalene derivatives, a 5% *n*-butanol–0.020 *M* SDS mobile phase was used to attempt the separation of toluene and chlorobenzene, which were not separated at a 0.035 *M* concentration of SDS. Fig. 6 shows the separation obtained under these conditions. Although the analysis time was longer because the retention of the compounds increased when the surfactant concentration decreased, a 0.020 *M* SDS concentration in the mobile phase allowed the separation of all the compounds in the mixture.

To study in greater depth the influence of the percentage of alcohol on selectivity, the effect of the nature and percentage of the alcohol on the efficiency obtained in MLC is currently being investigated.

CONCLUSIONS

For the benzene and naphthalene derivatives, SDS seems to give a better separation selectivity than CTAB and the separation selectivity generally increases when the surfactant concentration in mobile phase decreases. Medium-chain alcohols such as *n*-propanol and *n*-butanol positively influence the separation selectivity of the mixture studied, but *n*-butanol also shortens the analysis time. In the experimental separations, better selectivity was obtained with medium percentages of alcohol.

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