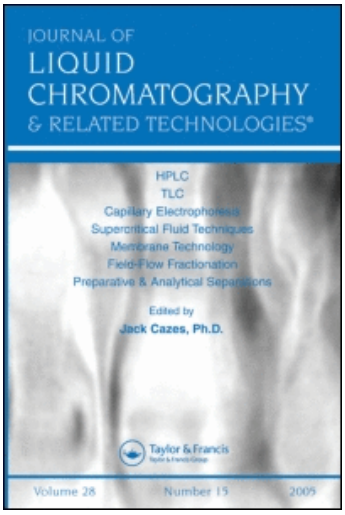

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SEPARATION STUDY OF PAHs BY HPLC USING A MICELLAR SDS MOBILE PHASE AND SHORT CHAIN COLUMNS

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ABSTRACT

The possibility of using micellar sodium dodecyl sulfate mobile phase modified with n-propanol to separate six PAHs on apolar columns was examined. The large capacity factors found in large-chain stationary phases made the analysis impractical. The use of short-chain stationary phases and the presence of n-propanol in the mobile phase, as a modifier, significantly decreased the capacity factors but also decreased resolution, allowing separation of five PAHs in reasonable analysis times. Conditioning of the column was easy and reproducible but the effect of temperature was quite critical. The gradient technique decreased peak width significantly.

INTRODUCTION

The importance of polycyclic aromatic hydrocarbons (PAHs) in environmental studies is well known.[1,2]

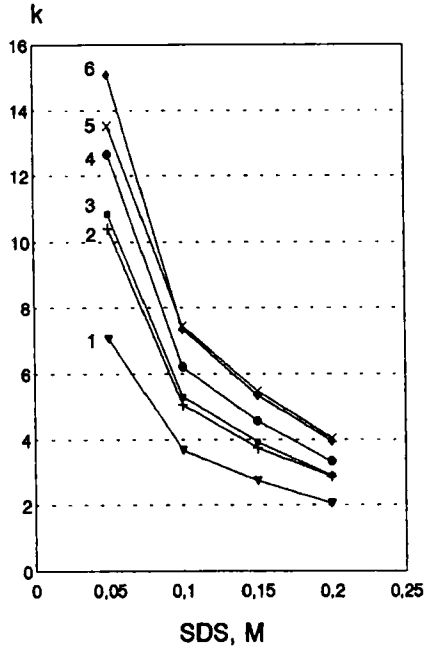

Figure 1. Effect of SDS concentration on capacity factor of PAHs. Conditions: C_4 column; flow-rate, 1 mL/min; temperature 600C; PAHs: 1,naphthalene; 2,acenaphthene; 3,phenanthrene; 4,pyrene; 5,benzo(a)anthracene; 6,chrysene.

Table 1

Characteristics of the Columns

Characteristics	C_{18}	C_4	C_1
Pore size Å	100	300	80
Surface area, m^2/g	350	50	200
Carbon load percentage, %	14	2.0	3.0
Calculated bonded phase coverage, $\mu mol/m^2$	2.06	4.8	4.8
End capping	Yes	Yes	No

The analytical techniques most often used for their determination are gas chromatography (GC) and, particularly, reverse phase high performance liquid chromatography RP-HPLC with fluorimetric detection. The sensitivity of the

latter technique can be increased by a suitable choice of excitation and emission wavelength pairs.[3] Additionally, the sensitivity and selectivity of fluorimetric determination of PAHs increases in micellar solutions.[4,5] Several authors claim that the use of micellar liquid chromatography (MLC) has advantages such as greater selectivity and sensitivity as well as lower toxicity and cost.[6-8] Very few works have been published regarding the separation of PAHs by MLC. Kord and Khaledi studied several, mainly polar, compounds by MLC.[9] Some information on the retention mechanism for non-polar compounds was supplied by Ji.[10] On the other hand, a mobile phase gradient acetonitrile/SDS allowed separation of eleven PAHs.[11] Nine PAHs were separated using a mobile phase (v/v), 0.05 M Brij-35/methanol: 50/50.[12] Solute-micelle association constants of some PAHs were calculated.[13,14]

Accordingly, the separation of PAHs by high performance liquid chromatography using micellar mobile phase (MLC) and short hydrocarbonated chains such as C_1 and C_4 was examined; organic modifiers were used in an attempt to shorten the high retention times in MLC reported in the literature for other compounds.[6,7,15-17]

EXPERIMENTAL

Apparatus and Material

The chromatograph consisting of a high pressure gradient Milton Roy CM 4000 pump, a Rheodyne 7125 sample injector with a 20 μL loop, a Waters 420 fluorimetric detector with the excitation and emission filters of 254 and 375 nm (long-pass), respectively, and a Milton Roy CI 4100 integrator. The columns were a C_{18} Nucleosil 5 μm particulate size (150 x 4.6 mm, Phenomenex) a C_4 Hypersil 5 μm particulate size (100 x 4.6 mm, Phenomenex) and a C_1 Ultremex 3 μm particulate size (100 x 4 mm, Phenomenex). Information about relevant characteristics of the columns are shown in Table I. A P-Selecta Precisterm bath was used for thermostating the columns. A P-Selecta Ultrasons bath was used for preparation all the solutions. A Lida nylon membrane filter with 0.45 μm pore size was used to filter the eluents used to prepare the mobile phase.

Chemicals

Standard stock methanol solutions of 6 PAHs (Sigma) at concentrations in the range 10^{-3}-10^{-4} M were used. More dilute solutions were prepared by dilution with methanol.

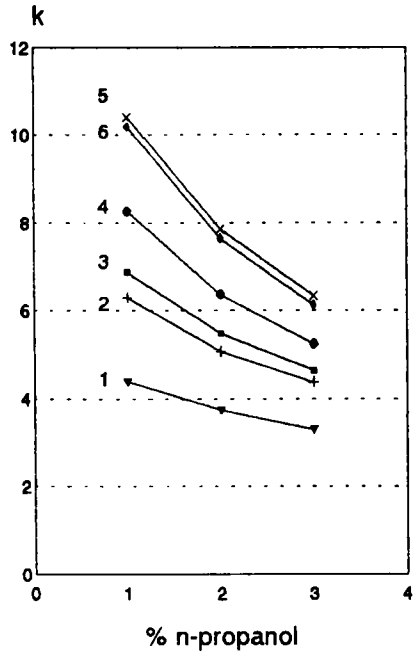

Figure 2. Effect of organic modifier on capacity factor.
Conditions: C_4 column; 0.10 M SDS mobile phase; flow-rate, 1 mL/min; temperature 600C; PAHs are identified in Figure 1.

An aqueous micellar solution of sodium dodecyl sulfate (SDS) ($C_{12}H_{15}NaSO_4$, FW = 288.38-Fluka) was prepared by stirring in an ultrasonic bath to give a final concentration of 0.20M, higher than its critical micellar concentration (CMC) = 8.1 x 10^{-3} M. More dilute solutions were prepared by dilution with water.

Methanol, n-propanol and n-butanol (Carlo Erba) of chromatographic grade were used. Water was obtained from a Milli-Q system (Millipore). All chemicals were of analytical reagent grade. Before use, all eluents were degassed under vacuum and filtered.

Procedure

In the isocratic mode micellar mobile phases (containig a surfactant concentration in the range of 0.05 M to 0.20 M) were used with small

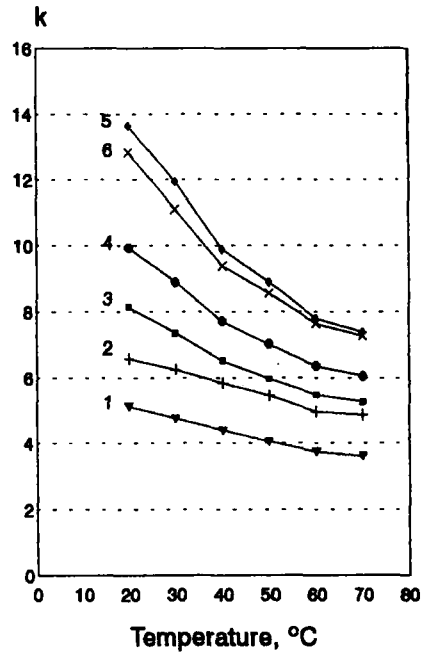

Figure 3. Effect of temperature on capacity factor.
Conditions: C_4 column; 0.10 M SDS with 2% n-propanol mobile phase; flow-rate, 1 mL/min; PAHs are identified in Figure 1.

percentages of n-propanol or n-butanol (in the range 1-3%) as organic modifiers at a flow-rate of 1 mL/min. In gradient chromatography the following gradient was used: 0.15 M SDS containing 12% n-propanol: water, 50:50 (v:v), changing to 0.15 M SDS containing 12% n-propanol in nine minutes, at a flow-rate of 1 mL/min.

Temperatures in the range 20-70°C were tested. Stock solutions of the PAHs were used and their concentrations were adjusted to allow detection in the range of ng/μL by the injection of 20 μL of standard sample. For fluorimetric detection excitation and emission filters of 254 nm and 375 nm (long-pass), respectively, were used. The column was conditioned by applying the following gradient: water for 15 minutes, which changed to 0.20 M SDS containing 2% n-propanol in 75 minutes, at a flow-rate of 1 mL/min. Acetone was used to determine the void time. C_1 Ultremex, C_4 Hypersil and C_{18} Nucleosil were tested stationary phases.

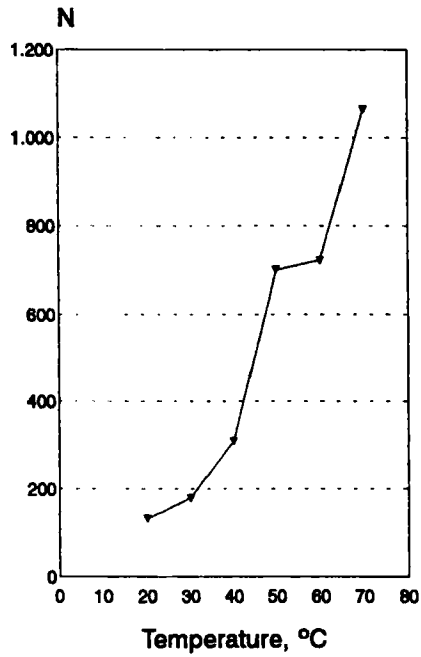

Figure 4. Effect of temperature on efficiency for pyrene. Conditions: C_4 column; 0.10 M SDS with 2% n-propanol mobile phase; flow-rate, 1 mL/min.

RESULTS AND DISCUSSION

Void Time Determination

Sodium nitrate was not appropriate to determine the void time, due to its electrostatic effects on the mobile phase, which contained an ionic surfactant.[16] Various compounds were tested for this purpose and acetone was found to give quite a significant signal due to the change in the refraction index; a 20 μL injection yielded a signal about twice that obtained from 20 μL 0.05 M sodium nitrate in conventional RP-HPLC with spectrophotometric detection.

Void times of 1.07, 1.14 and 1.36 minutes were found for the C_1 , C_4 and C_{18}, stationary phase, respectively, independently of the concentration of the surfactant. These void times were used for all k calculations.

Conditioning of the Column

Because the stationary phases C_1, C_4 and C_{18} are modified in the presence of the SDS[18-20] the column must be conditioned beforehand; this was carried out using water as a mobile phase for 15 minutes, which changed to 0.20 M SDS containing 2% n-propanol in 75 minutes at a flow-rate of 1 mL/min.

The column was thermostated at 60°C. Equilibrium was taken to be reached when the retention time for naphthalene remained constant, in the range 0.1 minutes. No column re-equilibration was necessary due to irreversibility of the SDS retention. The behaviour of C_1, C_4 and C_{18} columns did not change significantly for at least six-month working periods.

Effect of Surfactant Concentration

Figure 1 shows the effect of SDS concentration on capacity factors (k) of PAHs in the C_4 column. Clearly, retention decreased significantly with increasing SDS concentration, due to Micelle concentration elevation. According to the literature[21] k varies inversely with Micelle concentration. Resolution of the six PAHs studied is possible at low SDS concentrations; the best resolution or maximum spread of k values was obtained at 0.05 M SDS concentration. Using a mobile phase containing 0.20 M SDS there is evident overlapping and only four peaks can be observed. When six PAHs were injected, acenaphthene overlapped with phenanthrene, and benzo(a)anthracene with chrysene. The separation of isomers pair, benzo(a)anthracene-chrysene, is only possible using specific column.[3] Overlapping was higher with the C_1 column at the same SDS concentration in the mobile phase. Capacity factors with C_{18} column were very high even in the presence of 20% n-propanol as modifier, giving rise to impractical analysis times.

A reversal of capacity factor of the benzo(a)anthracene-chrysene pair was observed for SDS concentration form 0.10 M using the C_4 column.

Effect of the Organic Modifier

Low concentrations of organic modifiers are used to modify the surface of the stationary phase and provide the wetting needed for good mass transfer.[16] Alcohols such as n-propanol and n-butanol were tested as modifiers. The highest effect was found with n-butanol. The effect of n-propanol on k is shown in Figure 2, where 0.10 M SDS was used. Increasing percentages of the

modifier decreased k values but increased overlapping of the peaks; five peaks were observed in 2% n-propanol mobile phase. The same effect was found using n-butanol but half concentration was neccesary; however, n-propanol was chosen because resolution was better.

Effect of Temperature

Figure 3 shows the effect of temperature on capacity factors for six PAHs mixture. The capacity factors for all the PAHs decreased significantly with increasing temperature, the slope of these changes being higher than those found in RP-HPLC.[7,15,16] The decrease of the mobile phase viscosity with temperature improved the mass transfer, which decreased the C term in the Van Deemter equation. Consequently, the theoretical plate height decreased with the temperature, increasing the efficiency of column. In addition increasing the temperature should enhance the micellar kinetics. A temperature of 70°C was tried, but the column deteriorated clearly after a few runs. Figure 4 shows the effect of temperature on the theoretical number of plates, N, for pyrene. The effect of temperature is critical except in the range 50-60°C. This behaviour is similar for other PAHs. Consequently, a temperature in this range could be recommended.

The sharp decrease in pressure with rising temperature (Figure 5) is another beneficial effect of working at above room temperature. This is due to the decrease of viscosity in the mobile phase. This effect is higher than in conventional RP-HPLC, where changes in pressure drop with temperature are lower.

Effect of Stationary Phase

As indicated above, capacity factors on a C_{18} column were very high; e.g. for naphthalene the value of k was 15 with a mobile phase 0.20 M in SDS; in these conditions k values for naphthalene on C_4 and C_1 columns were 2 and 2.8, respectively, which is due to the carbon load percentage higher of the C_{18} column. In the presence of n-propanol in the mobile phase k values decreased significantly.

On the other hand, as it is shown in Figure 6, the best resoluton is obtained with the C_4 column, using 0.10 M SDS with 2% n-propanol as mobile phase.

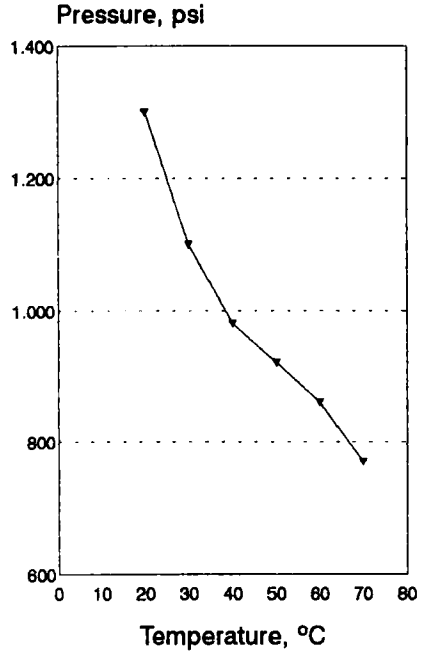

Figure 5. Effect of temperature on pressure.

Gradient Elution Technique

Because the PAHs peaks are quite close in isocratic conditions, gradient elution was tried to increase resolution, thus decreasing the width of the peaks.

The best results were found using a gradient starting with water: 0.15 M SDS and 12% n-propanol, 50:50 (v:v), changing to 0.15 M SDS and 12% n-propanol in nine minutes, at 60°C with a flow-rate of 1 mL/min. Although the decrease in peak width was significant in all the cases, five PAHs were resolved (Figure 7).

Different flow-rate gradients were also tested; starting with 1 mL/min and changing to 0.7 mL/min in 4 minutes gave a separation similar to that found with the concentration gradient, and the same five peaks appeared.

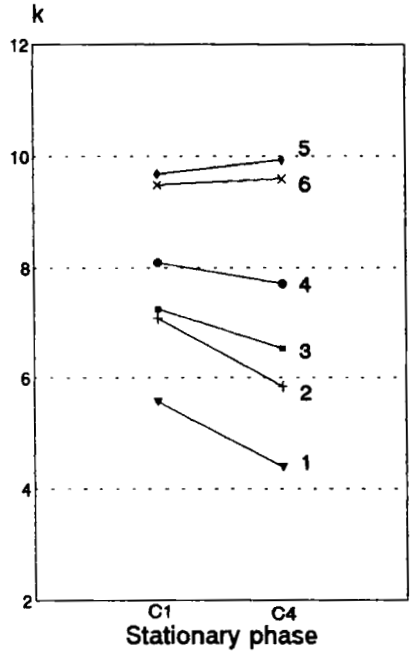

Figure 6. Effect of stationary phase on capacity factor. Conditions: 0.10 M SDS with 2% n-propanol mobile phase; flow-rate, 1 mL/min; temperature 40°C; PAHs are identified in Figure 1.

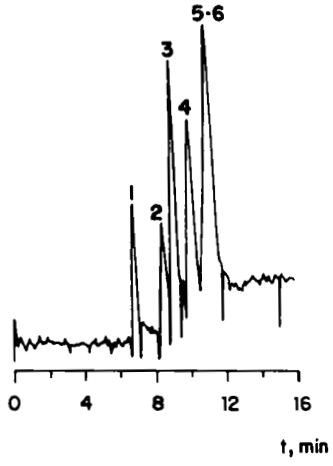

Figure 7. Separation of 6 PAHs using gradient elution.

CONCLUSIONS

The temperature effect is very important in MLC to improve mass transfer, which is an important problem in this technique. It is necessary to control analytical column temperature in the range 50-60°C to improve the PAHs separation.

The use of short alkyl bonded phases and the organic modifiers in the micellar mobile phase provide a more mass transfer and the wetting problem is be less severe.

In conclusion, the use of short columns as well as the addition of alcohols as organic modifiers in the mobile phase and the high temperature of analytical column are necessary for the PAHs separation by MLC.

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REFERENCES

1. S. E. Manahan, **Environmental Chemistry**, Lewis Publishers, Michigan, 1991.

2. C. Baird, **Environmental Chemistry**, W.H. Freeman and Company, New York, 1995.

3. M. N. Kayali, S. Rubio-Barroso, L. M. Polo-Dìez, J.Chromatogr.Sci., **33**, 181-185 (1995).

4. S. Rubio-Barroso, M. N. Kayali, L. M. Polo-Dìez, Química Analítica, **12**, 187-191 (1993).

5. S. Rubio-Barroso, M. N. Kayali, L. M. Polo-Dìez, Anal. Chim. Acta., **283**, 304-308 (1993).

6. J. S. Landy, J. G. Dorsey, J.Chromatogr. Sci., **22**, 68-70 (1984).

7. J. S. Landy, J. G. Dorsey, Anal. Chim. Acta., **178**, 179-188 (1985).

8. D. W. Armstrong, W. L. Hinze, K. H. Bui, H. N. Singh, Anal. Lett., **14 (A19)**, 1659-1667 (1981).

9. A. S. Kord, M. G. Khaledy, Anal. Chem., **64**, 1894-1900 (1992).

10. S. Ji. Fenxi Huaxue., **13 (9)**, 660-663 (1985).

11. M. N. Kayali, S. Rubio-Barroso, L. M. Polo-Díez, J.Liquid Chromatogr., **17 (17)**, 3623-3640 (1994).

12. D. Lopez-Lopez, S. Rubio.Barroso, L. M. Polo-Diez, J.Liquid Chromatogr., **18(12)**, 2397-2425 (1995).

13. M. A. Rodriguez, M. J. Sanchez, V. Gonzalez, F. Garcia Montelongo Chromatographia, **38 (5-6)**, 342-348 (1994).

14. V. Gonzalez, M. A. Rodriguez, M. J. Sanchez and F. Garcia Montelongo. Chromatographia, **34 (11-12)**, 627-635 (1992).

15. P. Yarmchuk, R. Weinberger, R. F. Hirsch, J. L. Cline Love, J.Chromatogr., **283**, 47-60 (1984).

16. J. G. Dorsey, M. T. De Echegarey, J. S. Landy, Anal. Chem., **55**, 924-928 (1983).

17. E. Pelizzetti, E. Pramauro, Anal. Chim. Acta., **169**, 1-29 (1985) .

18. A. Berthod, J. G. Dorsey, Analusis., **16 (2)**, 75-89 (1988).

19. J. G. Dorsey, M. G. Khaledy, J. S. Landy, J. L. Lin. J.Chromatogr., **316**, 183-191 (1984).

20. A. Berthod, I. Girard, C. Gonnet, Anal. Chem., **58**, 1356-1358 (1986).

21. M. Arunyanart, L. J. Cline Love, Anal. Chem., **56**, 1557-1561 (1984).

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