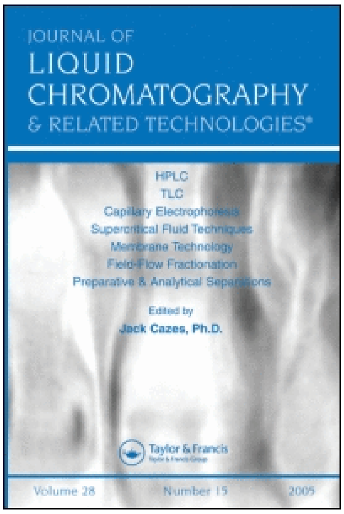

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SEPARATION AND IMPROVEMENT IN DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS BY REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING MICELLAR MOBILE PHASE AND FLUORESCENCE DETECTOR

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ABSTRACT

Separation and investigation of Detection Limit (LOD) of Polycyclic Aromatic Hydrocarbons ,PAH,compounds with RP-HPLC using fluorescence detection were studied . Lower LOD for these compounds were observed because of higher fluorescence intensity in the prescence of micellar mobile phase as compared to those of hydroorganics. The codition for optimum separation and enhancement of fluorescence intensity by variation of concentration of coorganic solvent (2-Propanol) and SDS were obtained. Selectivity factor and fluorescence intensity were the basic factors for the optimization of separation and improvement in detection respectively . The condition obtined were 3% of 2-Propanol in 0.035 M SDS.

INTRODUCTION

The PAH compounds are carcinogenic so that the identification and determination of these compounds in environment are very important .Micellar Liquid Chromatography ,MLC, with the use of fluorescence detector has a very good selectivity and sensitivity(1).The use of surfactant solution at concentration below critical micelle concentration ,CMC, as the mobile phase began in the beginning of 1970 and was named Ion-Pair Chromatoghraphy (2).Armstrong and Henry for the first time used the aqueous surfactant solution with concentration above CMC as the mobile phase in RP-HPLC which was named MLC (3,4) .

The advantages of micellar mobile phase over hydroorganic one are nontoxcicity , nonflammability ,its lower price and selectivity change because of increasing in the number of equilibrium.The higher fluorescence intensity of some compounds in micellar media as compared to hydroorganic mobile phases causes lower LOD with the use of fluorescence detector (5) .

The distribution of sample in hydroorganic mobile phase occurs between stationary and mobile phase while in MLC the sample molecule could be distributes between micelle-stationary phase,micelle-mobile phase and mobile -stationary phase, which could improve separation . The distribution of sample in micellar media are shown as the following :

$$\begin{tikzcd}
 & (E)_{mp} \arrow[dl, shift left, "K2"'] \arrow[dr, shift left, "K1"] & \\
(E)_M \arrow[ur, shift left] \arrow[rr, shift left, "K3"] & & (E)_{sp} \arrow[ul, shift left] \arrow[ll, shift left]
\end{tikzcd}$$

K1=distribution coefficient of sample between mobile and stationary phase
K2=distribution coefficient of sample between mobile phase and micelle
K3=distribution coefficient of sample between micelle and stationary phase
(E)mp=Representation of sample in aqueous mobile phase
(E)M= Representation of sample in micelle
(E)sp=Representation of sample in stationary phase

The capacity factor of the sample is related to the above coefficients and is shown by the following equation (6,7) :

$$1/k' = ([M]mp \cdot K2)/R.K1 + 1/R.K1 \qquad (1)$$

Where R is a constant its value depending on the amount of phase ratio.

Equation (1) shows that there is a linear relationship between 1/K' vs micelle concentration [M]mp . The value of K2 can be obtained by dividing intercept to slope of this line. The value of K2 represents the tendency of the sample for interaction with micelles . Since the value of K1 for nonpolar compounds is very high, equation (1) can be written as the following(8) :

$$1/K' = [M]mp/R.K3 \qquad (2)$$

$$K3 = K1 / K2$$

Micellar media can increase the fluorescence intensity because the quenching of the excited molecule by collision is decreased (9) . The reports show that efficiency of micellar mobile phase is lower than hydro-organic mobile phase (10) .To overcome this problem a coorganic solvent such as 2-PrOH was added to micellar mobile phase and column held at the temperature of 40°c.

In this project separation and improvement of detection of some PAH compounds by MLC were done with optimization of coorganic solvent and surfactant concentration.

EXPERIMENTAL

The PAH compounds used were from Fluka Company.In some case these compounds were purified by sublimation and recrystallization. The stock solutions were prepared in methanol with concentration range of 100-400 ppm . De-ionized double distilled water that was filtered by 0.45μm filter for the preparation of mobile phase .The needed mobile phase was made by dilution of 0.400M SDS solution with water and coorganic solvent . The HPLC pump were model 6000A with UV detector model 440A and fluorescence detector model 420-Ac all from Waters Company (Waters Assoc. Inc., Milford , MA).

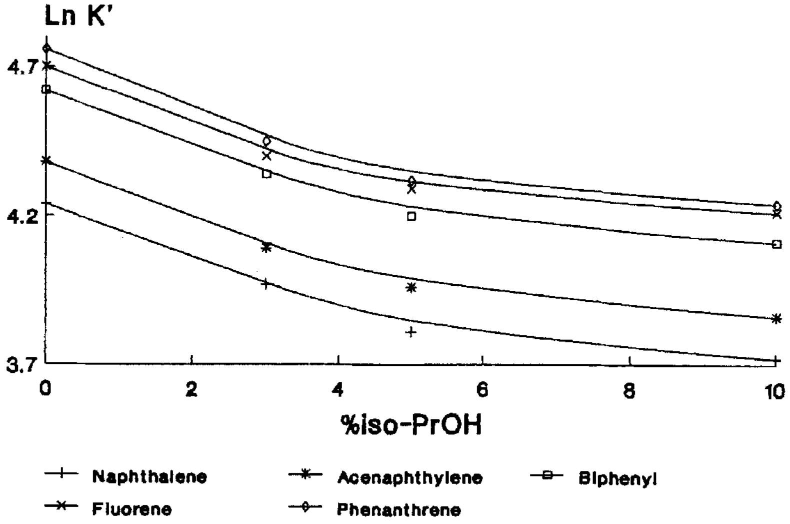

FIGURE 1. Effect of iso-PrOH percentage on K'

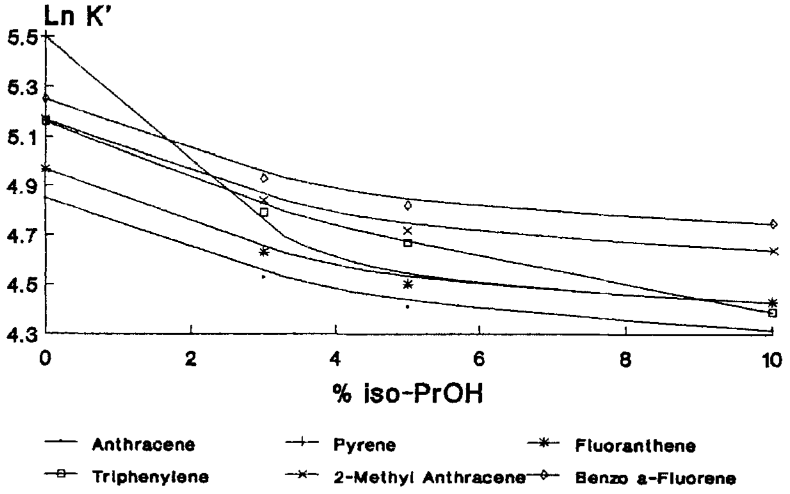

FIGURE 2. Effect of iso-PrOH percentage on K'

RESULTS and DISCUSSIONS

A)Effect of coorganic solvent on resolution

Effect of coorganic solvent in mobile phase on K' for PAH compounds were shown in fig. 1 & 2 . The results show a decrease in K'values with increase in concentration of coorganic solvent in mobile phase .The value of selectivity factor decreases as the percentage of coorganic solvent increases in mobile phase for all PAH compounds except for Triphenylene and 2-Methyl-Anthracene as shown in fig.3 . The viscosity and assymetric factor increases at higher concentration of 2-Propanol in mobile phase . The minimum amount of coorganic solvent needed for improvment in efficiency was about 3% and obtined experimentally.

B) Effect of SDS concentration on K' and selectivity factor

I) Relationship between K'and SDS concentration

Results shown in table 1 and 2 represent a linear relation between 1/K' vs SDS concentration .The distribution coefficient of sample between mobile phase and micelle (K2) derived from equation (1) by dividing slope to intercept of this line . The value of K2 can be only measured for Acenaphthene because of its lower hydrophobicity as compared with the other PAH compounds ,which they have negative intercepts . The negative intercept is related to the tendency of direct transfer of sample molecule from micelle to stationary phase.In these cases equation (2) gives better result and 1/K'vs surfactant concentration is quite linear.

II) Effect of SDS concentration on selectivity factor

The selectivity factor between adjucent peaks decreases for all PAH compounds (except for compounds 4,5

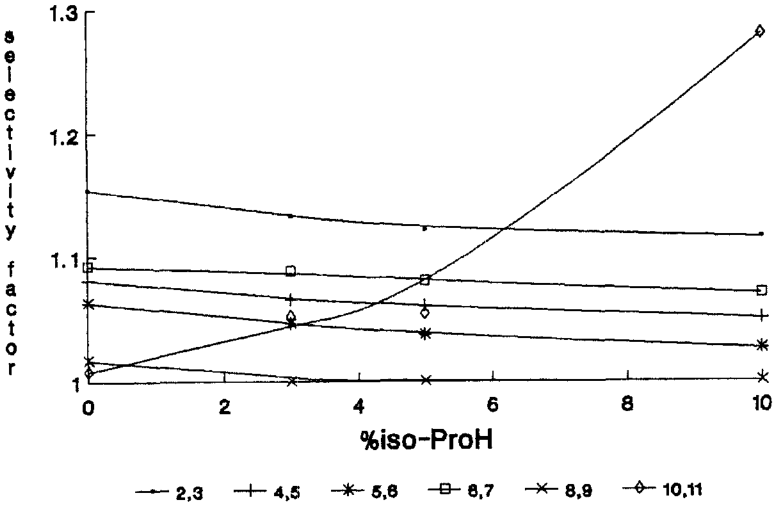

FIGURE 3. Effect of iso-PrOH percentage on selectivity factor

TABLE 1. Parameters Obtined From curve of 1/K' vs [SDS]
r=correlation coefficient , Int.=Intercept

Sample	Acenaph thene	Naphth alene	Acenaph thylene	Biphenyl	Fluorene	Phenan threne
Code	1	2	3	4	5	6
r	0.99	0.99	0.99	0.99	0.99	0.98
Slope	2.1	0.42	0.37	0.34	0.30	0.30
Int.	0.037	-0.0032	-0.0026	-0.0023	-0.0013	-0.0015
K2	56	-	-	-	-	-

TABLE 2. Parameters Obtined From curve of 1/K'vs [SDS] r=correlation coefficient, Int.=Intercept

Sample	Anthra cenee	Pyrene	Fluora nthene	Triphe nylene	2-Methyl Anthracen	11H-Benzo aFluorene
Code	7	8	9	10	11	12
r	0.99	0.98	0.99	0.99	0.99	0.98
Slope	0.28	0.26	0.26	0.23	0.22	0.20
Int.	-0.0013	-0.0021	-0.0021	-0.0024	-0.0016	-0.0018
K2	-	-	-	-	-	-

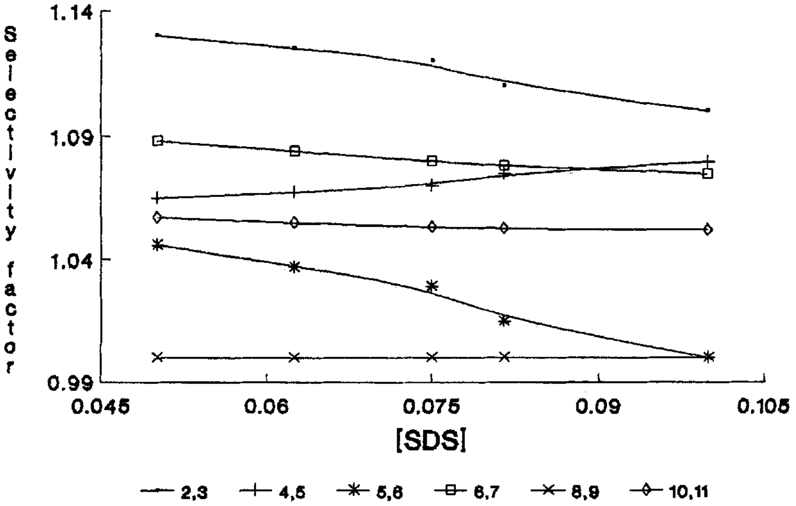

FIGURE 4. Effect of SDS conc. on selectivity factor

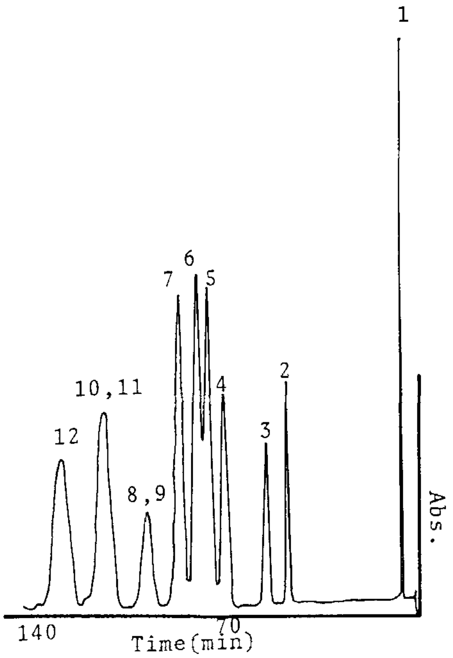

FIGURE 5. Typical chromatogram for separation of PAH compunds .
HPLC conditions : 0.035M SDS,%3 iso-PrOH(v/v) as MP with flow rate of 1 ml/min. , colum (3.9*150 mm) C18 (sample number as shown in tables 1 & 2)

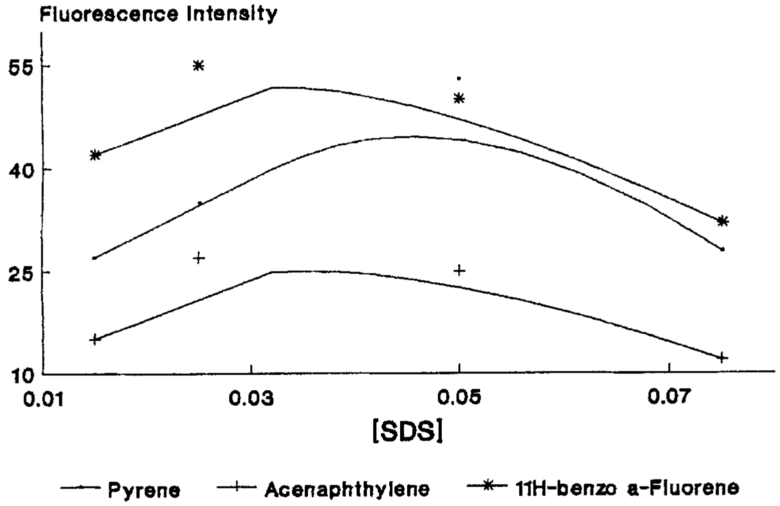

FIGURE 6. Effect of SDS consentration on fluorescence intensity

TABLE 3. The Comparison of LOD for Some PAH Compounds in Micellar and Hydroorganic Mobile Phase

Sample	Em.Filter (nm)	Ex.Filter (nm)	LOD 0.035M SDS	LOD 70/30 H2O/MeOH
Pyrene	400	338	1.74 ng/ml	17.4 ng/ml
Acenaph thylene	425	360	0.1 ppm	0.27 ppm
11 H-Benzo a-Fluorene	425	360	0.27 ppm	0.48 ppm

and 8,9) as the concentration of SDS increases in mobile phase (fig.4). The overall resolution at the lower SDS concentration increases at the expense of higher time of analysis. To improve separation of PAH compounds both SDS concentration and flow rate of mobile phase separately were changed.The experimental results show that the best resolution could be achieved with 0.035M SDS at 1.0 ml/min flow rate (fig.5).

III) The effects of surfactant concentration on fluorescence intensity

Effect of SDS concentration on fluorescence intensity of PAH compounds where their suitable absorption and emmission filter were available were studied . The fluorescence intensity vs SDS concentration as shown in fig.6 passes a maximum point . The lower fluorescence intensity before maximum point is related to the smaller ratio of number of micelles to sample molecules while after maximum it is due to impurities which quench the excited molecules. The maximum fluorescence intensity could occur where the number of micelles and sample are equal. The comparison of LOD in micellar mobile phase with hydroorganic mobile phase for some PAH compounds at same retention time is shown in table 3 . The results show a lower LOD for micellar mobile phase as compared to hydroorganic one.

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