

SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS WITH MIXED MICELLAR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Separation of Polycyclic Aromatic Hydrocarbons was performed by RP-HPLC using mixed micellar mobile phase. Effect of temperature, types and amounts of co-organic solvent in mobile phase on efficiency, asymmetry factor and selectivity factor showed that the appropriate condition for separation of these compounds was 3% iso-propanol (V/V) as co-organic solvent in mobile phase at 40°C. Analysis of the tri-dimensional plots of variation of SDS and Brij-35 concentration on selectivity factor showed that the optimum conditions were 45-65 mM of SDS and 1-1.5 mM of Brij-35 in micellar mobile phase.

Introduction

The separation and identification of Polycyclic Aromatic Hydrocarbons, PAH, are very important because of their carcinogenic effects [1]. Studies have shown the presence of these compounds in the environment (water, air and soil) [2,3], while many others have been carried out to identify their presence in coal tar, sediments and sedimentary rocks, water and marine organisms, air pollutants, etc. [4-9].

One of the most common methods for the separation and determination of these compounds is High Performance Liquid Chromatography, HPLC. Separation of PAH compounds with HPLC using C_{18} stationary phase was reported in 1971 for the first time [10]. The high selectivity of RP-HPLC is one of the important

reasons these compounds are analysed by this method [11].

The basic principle for selective separation in HPLC is due to control of interaction of solute between mobile and stationary phase by variation of mobile phase composition. Higher selectivity can be achieved by addition of a special chemical reagent to produce secondary chemical equilibria. One of these methods is addition of surfactants to mobile phase in HPLC [12]. If the concentration of surfactant in mobile phase is lower than critical micelle concentration, CMC, this technique is named ion-pair or soap chromatography [13]. The use of a surfactant solution with a concentration above CMC as a mobile phase in Reversed Phase High Performance Liquid Chromatography, RP-HPLC, was reported by Henry and Armstrong in 1980 for the first time and was named Micellar Liquid Chromatography, MLC, [14].

Micelles are formed from dynamic aggregation of surfactant monomers above CMC, and can produce

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different sites for interaction with hydrophilic and hydrophobic solutes. Types and concentration of surfactant control the selectivity and strength of mobile phase. In 1981, Armstrong *et al.* investigated the distribution of solute in micellar mobile phases [15, 16]. The efficiency of MLC was increased by the addition of an organic modifier to the micellar mobile phase by Dorsey *et al.* in 1983 [17].

The relationship between capacity factor and micelle concentration was reported by Cline Love *et al.* in 1984 [18] and at the same time gradient elution in MLC was also reported [19]. Separation and improvement in detection of PAH compounds in MLC were studied using fluorescence detection [20]. The existence of two different surfactants in mobile phase produces mixed micelles and the method is named Mixed Micellar Liquid Chromatography, MMLC.

The formation of mixed micelles in aqueous solution was studied by Schike and Manjing for the first time. The CMC of mixed micelle could be determined by breakness in the plot of a physical property (example surface tension) versus total concentration of surfactants. As we know, the formation of micelle with ionic surfactant is a result of balancing between van der Waals and electrostatic forces, whereas the formation of micelle with non-ionic polyoxyethylenic surfactant is the result of balancing the hydrocarbonic and polyoxyethylenic chains attraction forces. The extent of balancing forces and the CMC in mixed micelles depend on the mole percentage of surfactants in the solution [21].

The important factors influencing the formation of mixed micelle and its size are as follows:

- 1- The repulsive interaction between hydrophilic part of surfactant.
- 2- The hydrophobic interaction between the hydrophilic chain of surfactant and water.
- 3- The electrostatic interaction between the ionic parts of surfactant.
- 4- The conformational effects resulting from the penetration of hydrocarbonic chain into the micelle.
- 5- The entropy change resulting from the mixture of two surfactants in mixed micelle.

In this project, a solution of two different surfactants (SDS/Brij-35) was used as a mobile phase for separation of PAH compounds.

Experimental Section

Apparatus

The chromatographic measurements were carried out with HPLC system equipped with a series 10 LC pump, series 10 solvent programmer and LC-95 UV/Vis spectrophotometric detector, all from Perkin-Elmer

(Norwalk, CT, USA).

The column used was a 5 micron particle size Nova Pack C₁₈ (150 * 3.9 mm) from Waters Co. (Waters Assoc. Miliford, MA., USA). The column was thermostated at 40°C by a water circulator bath. The column dead volume was measured by multiple injection of methanol.

Reagents

Fluorene, phenanthrene, biphenyl, acenaphthylene, sodium dodecyl sulfate (SDS), polyoxyethylene (23) dodecanol (Brij-35) were all purchased from the Fluka company (Buchs, Switzerland). Acenaphthylene, naphthalene, propanol, iso-propanol, HPLC grade methanol and acetonitrile were obtained from the Merck company (Darmstadt, Germany).

The stock solutions of PAH compounds were prepared in methanol with a concentration range of 100-400 ppm. De-ionized doubly distilled water was filtered by 0.45 micrometer filter and used for preparation of the mobile phases.

Results and Discussion

I- Temperature Effect and Its Improvements

The temperature of the column affects pressure, analysis time and separation. The lower efficiency of MLC as compared to conventional hydroorganic mobile phase is due to higher viscosity of micellar mobile phase. Efficiency of column could be improved at higher temperatures due to faster mass transfer of solute between mobile and stationary phases [22]. The basic parameters for obtaining the optimum temperature were the number of theoretical plates, N, and asymmetry factor, B/A [23]. Figures 1 and 2 show the effect of column temperature on B/A and N respectively. It was concluded from these figures that 40°C was the most appropriate temperature for separation of these compounds.

II-Effect of Co-Organic Solvent in MLC

a) Choice of co-organic solvent

Here again two basic parameters N and B/A were used for selection of the best co-organic solvent (Table 1). The results showed that a considerable improvement in chromatographic efficiency was obtained by the addition of co-organic solvent to micellar mobile phase. The efficiency was increased in the following order for different co-organic solvents:

butanol > *iso - propanol* = *propanol* > *acetonitrile* > *methanol* > *water*

As can be seen, the efficiency increases as the polarity of the co-organic solvent decreases, and this could be related to the improvement brought about by wetting the stationary phase with the co-organic solvent. The highest efficiency was observed in the presence of butanol,

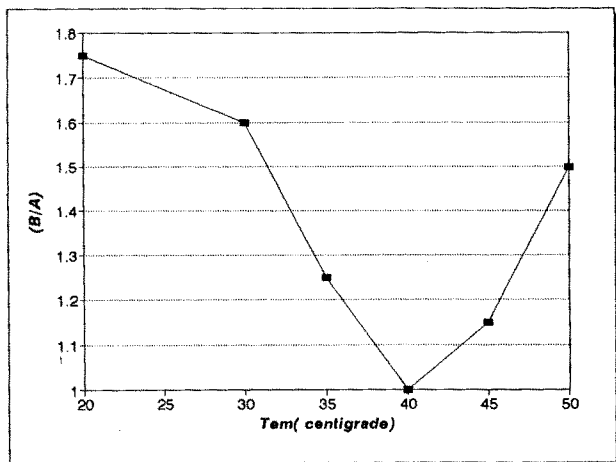


Figure 1. Effect of column temperature on asymmetry factor (B/A). Conditions as Table 1, micellar mobile phase has 3% of iso-PrOH.

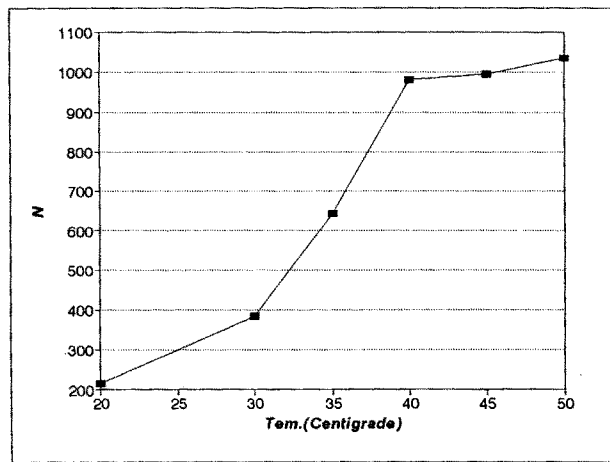


Figure 2. Effect of column temperature on the number of theoretical plates (N), conditions as Fig. 1

while the best peak shape was due to the presence of iso-propanol as co-organic solvent. Since efficiency in the presence of butanol was not much more than that of iso-propanol, the latter was selected as the co-organic solvent.

b) Determination of appropriate amount of iso-propanol in micellar mobile phase

To determine the amount of iso-propanol needed in mobile phase to effect an improvement in efficiency, the parameters N, B/A and selectivity factor were studied.

Table 1. Effect of types of co-organic solvents on asymmetry factor and number of theoretical plates.

Organic Solvent	t(min)	B/A	N
None	8.20	2.21	483
Acetonitrile	6.45	1.17	791
Methanol	7.00	1.24	725
Propanol	5.65	1.05	974
2-Propanol	5.60	1.00	980
Butanol	5.20	1.10	995

Conditions: column (3.9*150 mm) C₁₈ Nova Pack, thermostated at 40°C. Mobile phase was 1.5 mM Brij-35 and 60mM SDS with 5% (V/V) of organic modifier with flow rate 1 ml/min. Sample was 18 ppm acenaphthene.

The results show that the addition of more than 3% of iso-propanol did not have any effect on the improvement of efficiency and caused a change in the micelle structure, increased the mobile phase price and decreased the resolution of chromatographic separation [17,20].

III-Effect of SDS and Brij-35 Concentration on the Selectivity Factor

Increasing the concentration of SDS surfactant in the mobile phase increases the power of mobile phase and decreases the analysis time [24]. Consequently, at a higher concentration of SDS, the separation of PAH compounds will decrease. By addition of Brij-35 surfactant to SDS micellar mobile phase, mixed micelle will be formed and a decrease in CMC caused which results in an increase in the number of micelles as well as in the strength of mobile phase.

The molecules of Brij-35 surfactant are held strongly on the stationary phase and therefore the tendency of strongly hydrophobic molecules to interact with the stationary phase decreases and the retention time of PAH compounds is lowered. The effect of SDS and Brij-35 concentration on the selectivity factor is shown simultaneously in tri-dimensional curves (Figs. 3-6). These curves show that an SDS concentration of 45-65 mM with 1-1.5 mM of Brij-35 in mobile phase was the optimum concentration for separation of PAH compounds. The chromatogram obtained under optimum conditions is shown in Figure 7.

As shown in Figure 6, even though the selectivity factor for 2-methyl anthracene and 11 H-benzo (a)

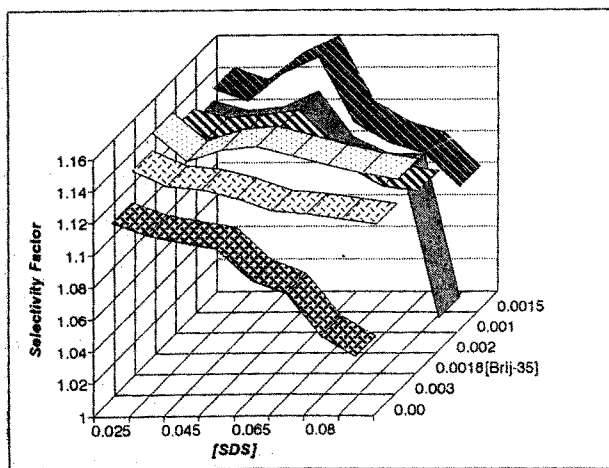


Figure 3. Effect of SDS and Brij-35 concentration on the selectivity factor of naphthalene and acenaphthylene

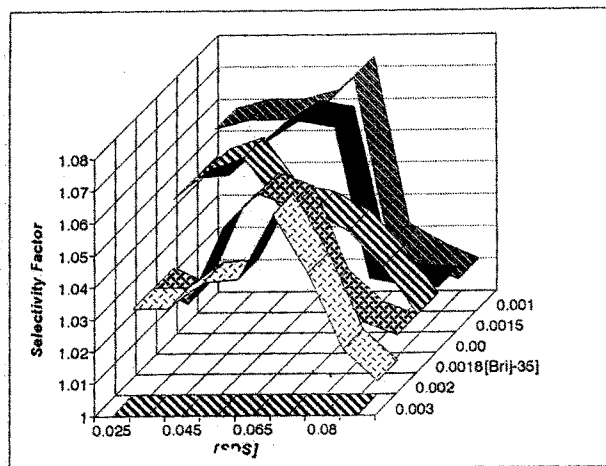


Figure 5. Effect of SDS and Brij-35 concentration on the selectivity factor of anthracene and fluoranthene

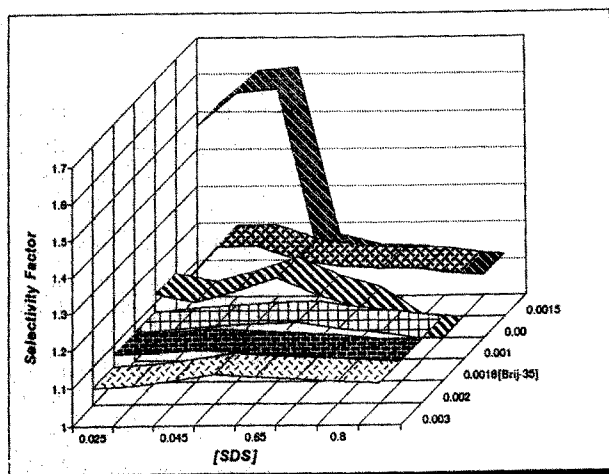


Figure 4. Effect of SDS and Brij-35 concentration on the selectivity factor of phenanthrene and anthracene

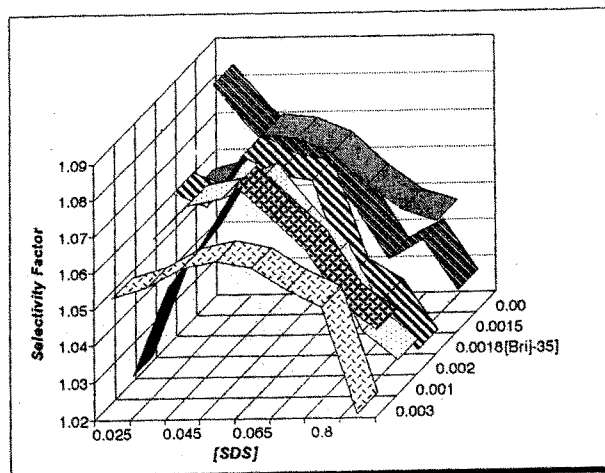


Figure 6. Effect of SDS and Brij-35 concentration on the selectivity factor of 2-methyl anthracene and 11 H-benzo (a) fluorene

fluorene with 25 mM SDS alone and 55 mM SDS + 1.0 mM Brij-35 in mobile phase was almost the same, the analysis time with mixed micellar was 30% of single micellar mobile phase (Figs. 7,8). The results show that mixed micellar mobile phase is better than single micellar mobile phase for separation of PAH hydrocarbons.

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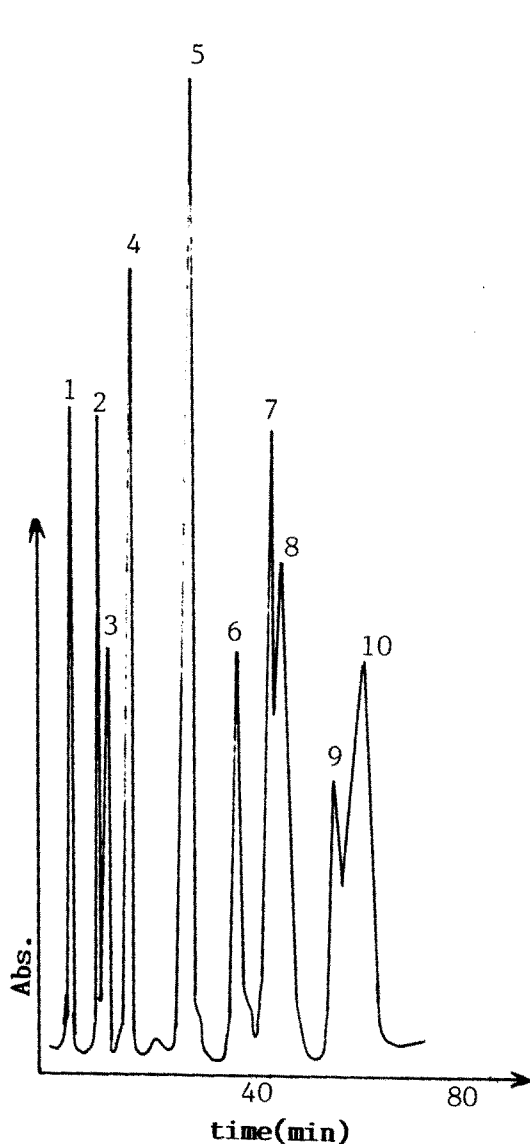


Figure 7. Typical chromatogram for separation of PAH compounds. HPLC conditions: 10 mM Brij-35, 45 mM SDS and 3% iso-PrOH(V/V) as mobile phase with flow rate 1.0 ml/min, column (3.4*150 mm) C₁₈ Nova pack, thermostated at 40°C. Samples are: Acenaphthene(1), naphthalene(2), acenaphthylene(3), biphenyl(4), fluorene(5), phenanthrene(6), anthracene(7), fluoranthene(8), 2-methyl anthracene(9) and 11 H-benzo (a) fluorene

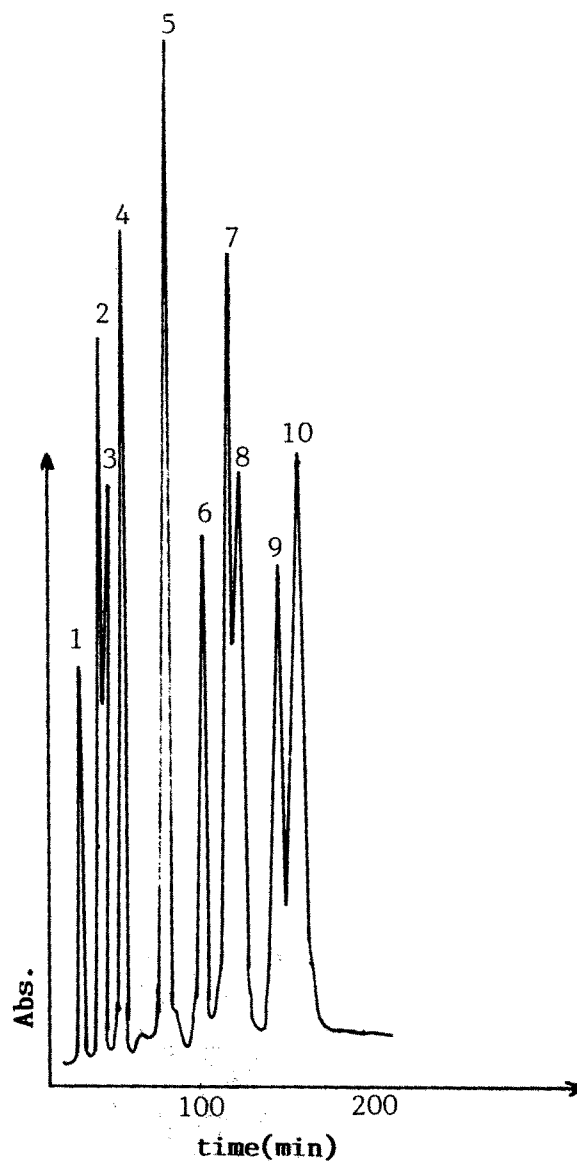


Figure 8. Typical chromatogram for separation of PAH compounds. HPLC conditions: 25 mM SDS, 3% iso-PrOH(V/V) as mobile phase, other conditions as Fig. 7.

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