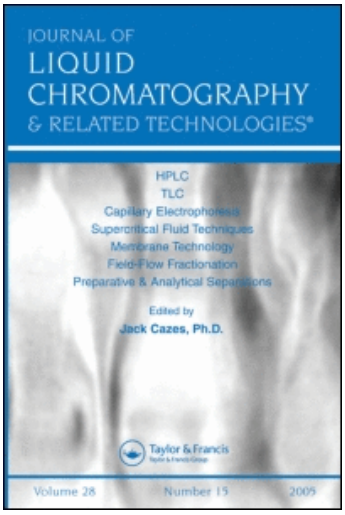

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D. Lopez-Lopez[a]; S. Rubio-Barroso[a]; L. M. Polo-Diez[a]
[a] Department of Analytical Chemistry Faculty of Chemistry, Complutense University, Madrid, Spain

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STUDY OF PAH'S SEPARATION AND PHASE-SOLUTE INTERACTION BY MICELLAR LIQUID CHROMATOGRAPHY

D. LOPEZ-LOPEZ, S. RUBIO-BARROSO*, AND L. M. POLO-DIEZ
Department of Analytical Chemistry
Faculty of Chemistry
Complutense University
28040 Madrid, Spain

ABSTRACT

The large k' values obtained in micellar liquid chromatography of Brij-35 on C-18 and C-8 columns clearly decrease in the presence of methanol or propanol as organic modifiers, propanol being superior to methanol although resolution is better with the latter. A linear relationship between k,' not log k', and the number of carbons is obtained. The distribution coefficient of the solutes between the stationary and aqueous phases increases, as do the micelle-solute association constants, with the number of aromatic rings for naphthalene, phenanthrene, chrysene and dibenzo(ah)anthracene. Sensitivity is increased with respect to that obtained in the classic isocratic RPLC methanol/water method. In best conditions nine PAH's are resolved to the baseline.

INTRODUCTION

Nowdays it is well accepted that micellar liquid chromatography (MLC) on alkyl-bonded stationary phases has some advantages over reversed phase liquid chromatography (RPLC);

these include the use of smaller amounts of organic solvents, which means less environmental pollution and lower cost as well as higher sensitivity for aromatic compounds by fluorimetric detection. Selectivity can also be increased by controlling additional chromatographic parameters such as the nature of the surfactant as well as its ionic charge and concentration[1-7]. However, there are some drawbacks, including greater broadening of the chromatographic peaks and larger capacity factors, which make analysis time impractically long. This effect is more important in RPLC when the polarity of the analytes decreases; stationary phases based on short hydrocarbonated chains decrease the capacity factors but they also decrease the resolution[7-9].

The behaviour of analytes in MLC arises from more complex interactions than in RPLC, making it difficult to predict the results in given chromatographic conditions. Consequently more data are required to probe further into this field of chromatography.

In this paper we study the behaviour of 13 polycyclic aromatic hydrocarbons (PAH's) in MLC, based on the use of Brij-35, a non ionic surfactant, using two alkyl-bonded stationary phases, C-8 and C-18, and fluorimetric detection. Some relevant thermodynamic data based on equations described in the literature[1-4,10,11] are applied to explain this behaviour. From the analytical point of view the results could also be useful for determining these compounds in environmental studies.

EXPERIMENTAL

Apparatus and material

The chromatographic system consisted of the following components: a high pressure gradient LDC Analytical 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 Hypersil 5 μm particle size (100x4.0 mm) and a C-8 Hypersil 5 μm particle size (100x4.6 mm). A P-Selecta Precisterm bath was used for thermostating the columns. A P-Selecta Ultrasons bath was used for preparing all the solutions. A Lida nylon membrane filter with 0.45 μm pore size was used to filter the eluents employed to prepare the mobile phase. Vapor pressure osmometry measurements were carried out on a Knauer VPO instrument.

Chemicals

Stock standard methanolic solutions of 13 PAH's from Sigma at concentrations in the range 10^{-3} - 10^{-4} M were prepared by weighing and dissolving the solid products in methanol. More dilute solutions were prepared by dilution with methanol.

An aqueous micellar solution of Brij-35 [polyoxyethylene 23 lauryl ether, C_{12} H_{25} $(OCH_2-CH_2)_{23}$ OH; formula weight = 1199.6; 30% (w/v)] from Sigma, was prepared by dissolving 200

ml in water up to 500 ml to give a final concentration of 0.1 M; critical micellar concentration (c.m.c.) = 10^{-4} M[12]. More dilute solutions were prepared by dilution with water, methanol/water or propanol/water, where appropriate.

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

Chromatographic procedure

Micellar mobile phases containing Brij-35 concentration in the range 4.10^{-4}-0.1 M were used; depending on the case, propanol or methanol in the ranges 2-30% and 20-80%, respectively, were used as organic modifiers at flow-rates of 1 and 0.6 ml/min, for C-18 and C-8 columns, respectively.

Stock solutions of the PAH's were used and their concentrations were adjusted to allow detection in the range $4.0.10^{-3}$-120 μg/ml for naphthalene and anthracene, respectively, by the injection of 20 μl of standard samples. For fluorimetric detection, excitation and emission filters of 254 and 375 nm (long-pass), respectively, were used.

Temperatures in the range 30-60ºC and flow-rates in the range 0.4-1 ml/min were tested.

The columns were conditioned by applying the following gradient: methanol for 15 minutes and then water for a further 15 minutes, which changed to the micellar Brij-35 solution

working concentration in 60 minutes at a flow-rate of 1 ml/min; this micellar solution was then maintained for a further 60 minutes. Acetone/water was used to determine the void times.

RESULTS AND DISCUSSION

Conditioning of the stationary phase

It is known that work in MLC requires prior conditioning of the column to achieve reproducible results. It is accepted that the column is modified by adsorption of the surfactant. When using the Brij-35 surfactant, the C-18 and C-8 columns are completely regenerated by passing pure methanol; consequently, the conditioning procedure specified in the experimental section was applied. Reproducibility of the naphthalene, which is the earliest-eluting solute, with a retention time in the range ± 0.1 min was taken as a measure of the stability of the column. Reconditioning of the column was necessary after a month's work, equivalent to 175 hours; in any case elution of the column with methanol for 120 minutes allows it to be used in RPLC without significant changes.

Determination of void volume

Different approaches have been used to determine the void volume in MLC. The uracil method[2,10] was not successful in this case despite using fluorimetric detection. Other methods based

on the change of refraction index, such as the use of water, urea, acetonitrile/water and deuterated water were also unsuccessful[3,7,10]. A new approach based on the use of acetone/water yielded good results, although acetone can be retained somewhat on the stationary phase. Injection of 20 μl of acetone/water 20/80 (v/v) gave a signal similar to the one obtained by injection of 20 μl of naphthalene 5.10^{-4} M; this signal is also based on a refraction index change. This method was also successful for SDS in MLC[5,9]. Under the typical working conditions (flow-rate 1 ml/min, Brij-35 concentration 0.05 M and temperature 60ºC) the void volumes were 0.80 ml and 1.08 ml for the C-18 and C-8 column, respectively. These values scarcely changed over the Brij-35 concentration range of 10^{-2}-10^{-1} M. It should be noted that the calculated bonded phase coverage in μ mol/m^2 was 2.84 and 3.85 for C-18 and C-8 columns, respectively.

Effect of the surfactant concentration on the mobile phase

A micellar mobile phase containing a Brij-35 concentration 4.10^{-4} M, which is close to its c.m.c., gave capacity factors for naphthalene above 61 and 26 on C-18 and C-8 columns, respectively. As shown in Figure 1, retention times (and, consequently, capacity factors) on a C-18 column decrease with increasing Brij-35 concentration in the mobile phase. However, at 0.05 M Brij-35, capacity factors are impractical from an analytical point of view. In these conditions, only five PAHs

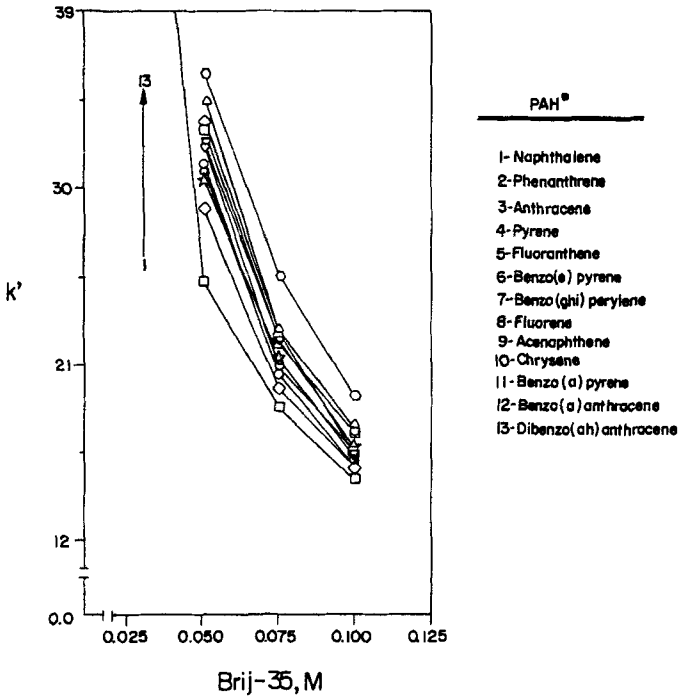


Figure 1: Capacity factors versus micellar concentration of Brij- 35. Flow-rate, 1 ml/min; temperature, 60ºC; detection, 254 nm excitation and 375 nm emission (long-pass); C-18 column. *Elution order in 0.05 M Brij-35.

are resolved to the baseline. Moreover, at 0.1 M Brij-35 only four PAH's are resolved. Similar changes were observed on a C-8 column, where capacity factors changed from the range 12-16.2 to 6.5-9.0 with the same Brij-35 concentration change. On the other hand, the elution order changed with Brij-35 concentration and with the column type (C-18, C-8). These changes are consistent with some thermodynamic parameters determined below.

Effect of the organic modifiers

A common practice to decrease capacity factors is based on addition of organic modifiers, such as methanol, propanol, butanol or acetonitrile[1-9]. An additional beneficial wetting effect is assumed for these modifiers. Methanol and propanol were tested, the elution strength of propanol being higher than that of methanol. Similar analysis times were obtained both for 30% propanol and 50% methanol, resolution being lower with propanol. As shown in Figure 2, capacity factors also decrease with methanol percentages. A change in the chromatographic mechanism for the larger PAH's is apparent in this figure. Capacity factors lower than 10 are obtained for methanol percentages above 60%, which makes the analytical separation practical. Eleven PAH's can be separated with 50% methanol at a flow-rate of 1 ml/min. Similar results were obtained with a C-8 column, changing log k' values from the range 0.7 - 1.0 to (-0.5) - (-0.2) for the change of methanol percentage from 30 to 60%. On the other hand, elution orders are independent of the column type and of the methanol percentages. Thus PAH's can be separated with 50% methanol and flow-rate of 0.6 ml/min.

Determination of the critical micellar concentration

After selection of the percentages of methanol, the critical micellar concentrations (c.m.c.) in these conditions were determined by vapor presure osmometry[13,14]. The results

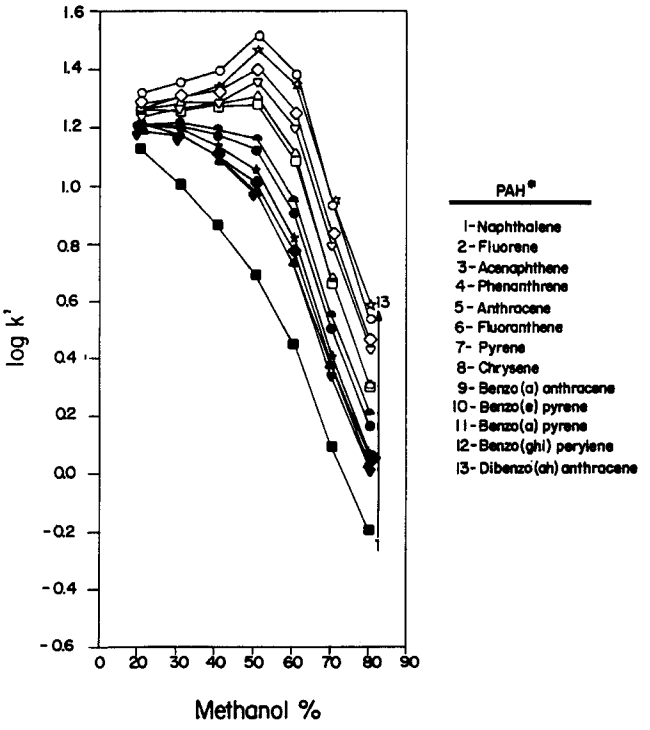


Figure 2: Capacity factors versus methanol concentration. Brij-35 concentration, 0.05 M; Flow-rate, 1 ml/min; temperature, 60ºC; detection, 254 nm excitation and 375 nm emission (long-pass); C-18 column. *Elution order in 50% methanol.

found are shown in Figure 3. Graph number 1 relates to glucose solutions taken as a reference and the graph number 2 to Brij-35 solutions. In both cases we started with a 50/50 v/v methanol/water solution and added glucose or Brij-35.

Graph 1 shows a regular increase of the resistence, ΔR, with glucose concentration; in contrast, the inflection point of graph 2 allows the c.m.c. of Brij-35 to be determined in 50%

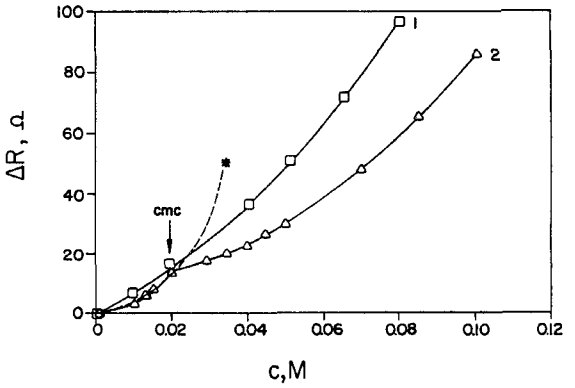


Figure 3: Determination of the critical micellar concentration. Methanol/water, 50/50 (v/v). 1, glucose solution; 2, Brij-35 solution; *Assuming the absence of micelles.

methanol. This c.m.c. value was 0.02 M, which is higher than that obtained in the absence of methanol, 10^{-4} M[12]. Obviously, lower methanol contents and a 0.02 M Brij-35 concentration will always give a micellar system. The existence of micelle structures in the presence of such a high concentration of organic modifiers could be questioned; however, results obtained seem to confirm that micelles exist in these experimental conditions.

Solvent strength

According to the literature[1,10] the solvent strength of the mobile phase decreases in the presence of micelles. We determined the solvent strength of the micellar mobile phases

with different percentages of methanol, using the equation[15] described elsewere:

$$\log k' = - S \theta + \log k_o' \qquad (1)$$

For a particular solute, plotting the logarithm of the capacity factor (k') against the volume fraction (θ) of methanol allows the solvent strength (S) to be determined from the slope. The intercept, log k_o', is the logarithm of the capacity factor in micellar mobile phase without a modifier.

As shown in Table 1, the solvent strength decreases with the molecular size of the PAH. This agrees with results for other compounds and for other micellar mobile phases in the presence of propanol as a modifier[10]. The range of variation is even lower for the C-8 column. Some PAH solvent strength values for a traditional methanol mobile phase on a C-18 column are included in this table for comparison purposes.

Effect of flow-rate

The effect of flow-rate on retention times for a C-18 column is shown in Figure 4. Retention times decrease with the flow-rate, and eleven PAH's can be separated with analysis times below 36 minutes for a flow-rate of 1 ml/min. The large retention times observed for flow-rates below 1 ml/min seem to indicate the important contribution of kinetic factors. Good resolution up to nine PAH's was obtained with the C-18 column.

TABLE 1

Solvent strength of the mobile phase in C-18 and C-8 columns

PAH	S, C-18		S, C-8*
	*	**	
Naphthalene	2.2	3.0	2.2
Fluorene	1.9		2.4
Acenaphthene	2.0		2.3
Phenanthrene	1.9		2.3
Anthracene	1.9		2.3
Fluoranthene	1.7		2.2
Pyrene	1.6	3.3	2.2
Chrysene	1.5		2.1
Benzo(a)anthracene	1.5		2.1
Benzo(e)pyrene	1.2	2.9	1.9
Benzo(a)pyrene	1.2	2.9	1.9
Benzo(ghi)perylene	1.0	2.8	1.7
Dibenzo(ah)anthracene	1.1		1.7

* Mobile phase, 0.05 M Brij-35 in water/methanol solution.
** Mobile phase water/methanol.

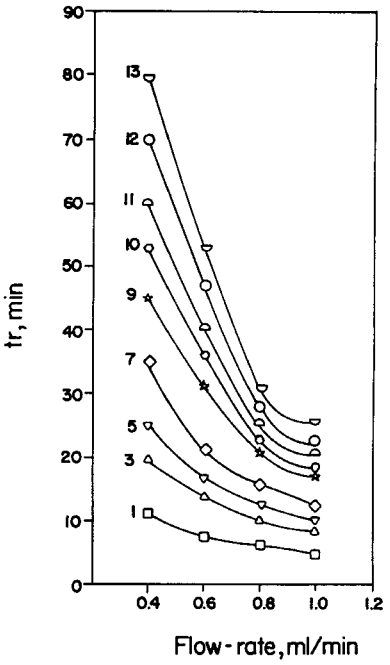


Figure 4: Effect of flow-rate on retention time. Brij-35 concentration, 0.05 M in methanol/water 50%; temperature, 60ºC; detection, 254 nm excitation and 375 nm emission (long-pass); C-18 column. PAH's identified in Figure 2.

The same behaviour was found for the C-8 column; separation of ten PAH's was achieved with a flow-rate of 0.6 ml/min.

Effect of temperature

The effect of temperature on MLC is greater than in RPLC[5,7-9]; the decrease in viscosity of the micellar mobile phase with increasing temperature reduces retention time significantly. The results obtained are shown in Figure 5, for

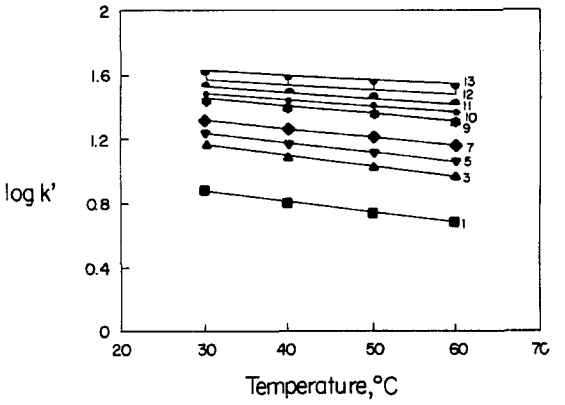


Figure 5: Effect of temperature on capacity factors. Brij-35 concentration, 0.05 M in methanol/water 50%; Flow-rate, 1 ml/min; detection, 254 nm excitation and 375 nm emission (long-pass); C-18 column. PAH's identified in Figure 2.

a C-18 column. The change in k' is higher for the PAH's with smaller molecular size. An increase of 10ºC produces a log k' change of 0.2 units. Again, similar changes were observed for a C-8 column.

Correlation between retention factors and the number of carbons

In RPLC there is a linear relationship between log k' and the number of carbons, Nc, as shown in the following equation[16,10]:

$$\log k' = (\log \alpha)\, Nc + \log \beta \qquad (2)$$

where the slope, log α, is a measure of hydrophobic selectivity which characterizes non-specific interactions; the intercept

indicates the specific interactions between the residue of the molecule and the mobile and stationary phases.

However in MLC a linear relationship between k' and Nc is obtained, as follows[10]:

$$k' = B\ Nc + A \qquad (3)$$

where A and B are the intercept and slope, respectively, of the straight line.

The results obtained for three Brij-35 micellar mobile phases are shown in Figure 6; straight line plots of k' versus Nc were also obtained for different methanol percentages in mobile phases, Figure 7. This confirms the results obtained for other homologous series[1,2,10]. It should be noted that the slope of the graph increases significantly with the presence of methanol, thus increasing resolution. These results are clearly different from those obtained with a 50% methanol/water mobile phase; in this case, only naphthalene is resolved, the capacity factor being 26.5.

Determination of the micelle-solute association constants and the distribution coefficients of the solute.

Several parameters have been proposed[1-3,10,11] in order to explain the above relationship between k' and Nc. The micelle-solute association constants per surfactant monomer (K_2) can be determined from the Armstrong and Nome equation[17]:

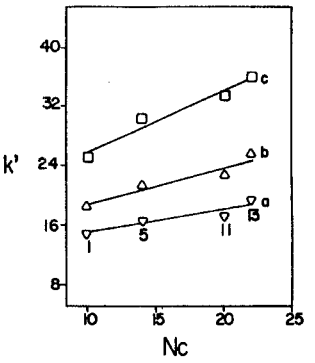


Figure 6: Correlation of capacity factors and the number of carbons. Mobile phase, Brij-35, M, a, 0.10; b, 0.075; c, 0.050. PAH's identified in Figure 2.

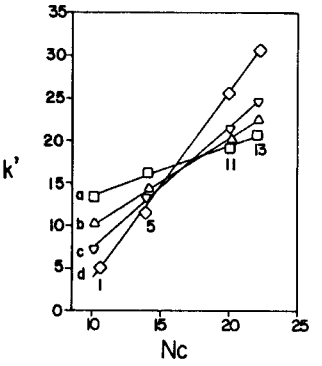


Figure 7: Correlation of capacity factors and the number of carbons. Mobile phase, Brij-35 0.05 M. Methanol, %, a, 20; b, 30; c, 40; d, 50. PAH's identified in Figure 2.

$$V_s/(V_e - V_m) = [V(P_{mw}-1)/P_{sw}]C_m + 1/P_{sw} \; ; \; K_2 = V(P_{mw}-1) \qquad (4)$$

The graph of the ratio of the stationary phase volume (V_s) to the difference between elution volume of the solute (V_e) and the void volume of the column (V_m) versus the micellized surfactant concentration (C_m), which is the surfactant concentration minus the c.m.c., gives rise to straight-line plots with slope K_2/P_{sw}; where P_{sw} is the distribution coefficient of the solute between the stationary and aqueous phases, which can be calculated from the intercept. V is the molar volume of the surfactant. P_{mw} is the distribution coefficient of the solute between the micellar and aqueous phases.

Similarly, the Arunyanart and Cline Love equation can be used for the same purpose[18]:

$$1/k' = [K_2/\Phi(L_s)K_1]\ C_m + 1/\Phi(L_s)K_1 \qquad (5)$$

where Φ is the ratio V_s/V_m, L_s is the concentration of stationary-phase ligate and K_1 the association constant for the solute between the bulk solvent and the stationary phase. In this case K_2 can be determined from the slope of 1/k' versus C_m; K_2 is obtained easily as a ratio between the slope and the intercept.

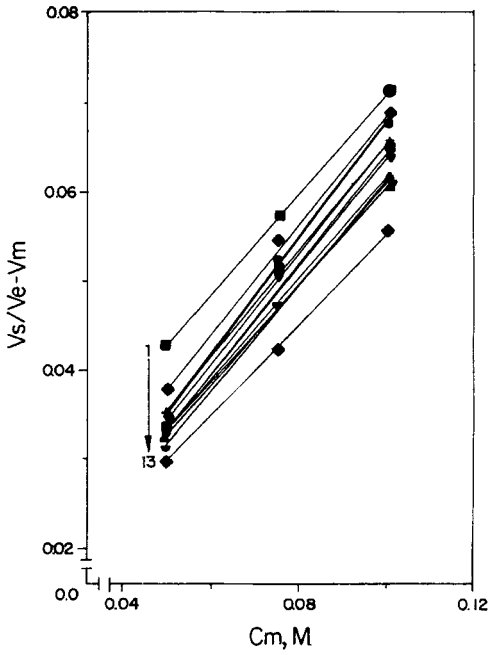


Figure 8: Effect of micellized Brij-35 concentration on the $V_s/(V_e-V_m)$ ratio. PAH's identified in Figure 1.

Figures 8 and 9 respectively show the variations of $V_s/(V_e - V_m)$ and $1/k'$ as a function of the micellized Brij-35 concentration. The results are shown in Table 2.

Related to K_2 is the solute-micelle association constant per micelle (K_s), which can be calculated by multiplying K_2 by the aggregation number, which is 40 for Brij-35[12]. Both the above equations yielded similar results for K_2. There is apparently no correlation between K_2 and molecular size of the solute; in fact the PAH's listed have different structures. However the slopes of the graphs in Figures 8 and 9 are not the

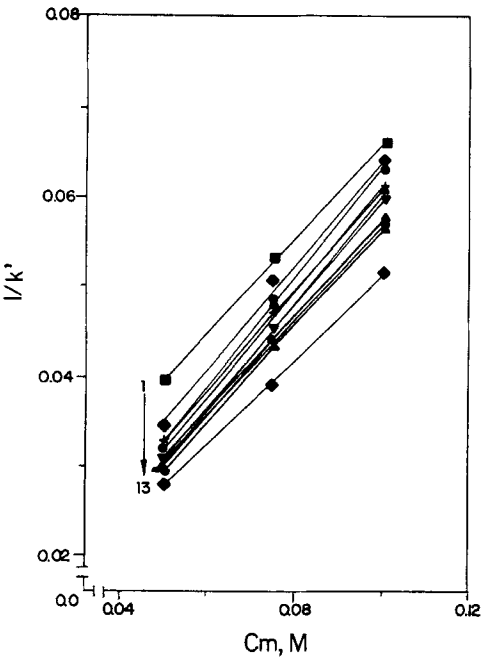


Figure 9: Variation of 1/k' versus the micellized Brij-35 concentration. PAH's identified in Figure 1.

same for all the PAH's studied, indicating that the effect of Brij-35 concentration differs with each PAH, as indicated before. Focusing on PAH's with similar structure, for two, three, four and five aromatic rings (e.g. naphthalene, phenanthrene, chrysene and dibenzo(ah)anthracene), increasing K_2 values were obtained for increasing numbers of rings in the linear ring structure; this agrees with results reported recently for Brij-35 MLC[19]. The size of the micelle cavities must be related to the behaviour of the different PAH's.

Similarly, there is apparently no correlation between the molecular size of all PAH's and the distribution coefficient of

TABLE 2

Binding constant calculations.

	Equation (4)			Equation (5)	
PAH	K_2,*10 (M^{-1})	K_S,*10^3 (M^{-1})	P_{sw}, *10	K_2,*10 (M^{-1})	K_S,*10^3 (M^{-1})
Naphthalene	4.0	1.6	6.9	4.0	1.6
Acenaphthene	10	4.1	19	11	4.2
Phenanthrene	9.3	3.7	15	9.5	3.8
Anthracene	12	4.8	20	12	4.7
Fluoranthene	39	16	59	39	15
Pyrene	32	13	48	31	12
Chrysene	13	5.3	23	13	5.2
Benzo(a)anthracene	33	13	56	31	12
Benzo(e)pyrene	23	9.4	37	26	10
Benzo(a)pyrene	20	7.9	33	20	8.1
Benzo(ghi)perylene	26	10	42	26	10
Dibenzo(ah)anthracene	14	5.7	23	14	5.6

the solute between the stationary and aqueous phases. However, for linear structures, as is the case of the four compounds mentioned above, increasing P_{sw} values have been obtained for increasing ring number. As before, when the straight-line plots

of $V_s/(V_e-V_m)$ or $1/k'$ versus C_m for two PAH's are parallel, the effect of C_m is similar for them.

Free energy of transfer from water to micellar phase

Another parameter used to show interactions between the solute and the micelle is the free energy of transfer from water to micelle, defined as:

$$\Delta \mu^{o}_{t}(w/m) = -RT \, Ln(55.5 \, K_2) \qquad (6)$$

where R and T are the gas constant and the temperature, respectively[20]. These energies are usually compared with those from octanol to water, which involves mainly hydrophobic interactions described by the following equation:

$$\Delta \mu^{o}_{t}(o/w) = -RT \, Ln(55.5 \, P) \qquad (7)$$

where P is the octanol-water distribution coefficient in molarity units, which can be calculated from

$$\log P = \log P_{o/w} - \log (MW)_o \, d_w/(MW)_w \, d_o \qquad (8)$$

where $(MW)_o$, $(MW)_w$, d_o and d_w are the molecular weight and density of octanol and water, respectively, and $P_{o/w}$, which is a measure of the hydrophobic nature of a solute, is expressed in molar fractions[11,21]. The results are shown in Figure 10. A

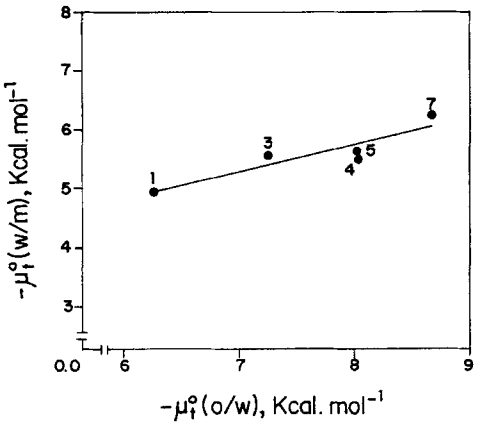


Figure 10: Variation of μ^{o}_{t}(w/m) versus μ^{o}_{t}(o/w). PAH's identified in Figure 2.

straight-line plot of μ^{o}_{t}(w/m) versus μ^{o}_{t}(o/w) was obtained. The variation of the transfer energy for octanol-water is higher than for water-micelle, whose slope is 0.46.

Correlation between binding constants and number of carbons

According to the literature[10], there is a linear relationship between the logarithm of the binding constant of the solute to micelle (log K_2) or the solute distribution coefficient between the stationary and aqueous phases (log P_{sw}) versus the number of carbons in the normal chain length; these relationships are defined by the following equations:

$$\log K_2 = a\ Nc + b \qquad (9)$$

$$\log P_{sw} = a' Nc + b' \quad (10)$$

where a is a measure of the free energy of transfer of additional carbon from the bulk solvent to the micelle, or to the stationary phase (a'); and b represents the interaction between the homologous rest with the micelle or stationary phase (b'). The results are shown in Figures 11 and 12. Clearly, plots of both log K_2 and P_{sw} versus Nc are linear for the compounds studied. The larger binding constant values for the homologous PAH's indicate the stronger interactions of the solutes with the Brij-35 micelles.

Correlation between transfer free energy from water to micelle and the number of carbons

The transfer free energy per mole of solute from water to micelle, $\mu^o_t(w/m)$, is related to the number of carbons, Nc, by the following equation[22]:

$$\Delta \mu^o_t(w/m) = Nc \Delta \mu^o_C + \Delta \mu^o_{Ar} \quad (11)$$

where the slope represents the free energy for each additional carbon and the intercept is the free energy of the rest of the aromatic structure. As shown in Figure 13, most of the PAH's studied fit a linear graph. The slope of the graph, i.e. the increase of the transfer free energy per additional carbon, is about - 90 cal mol^{-1}.

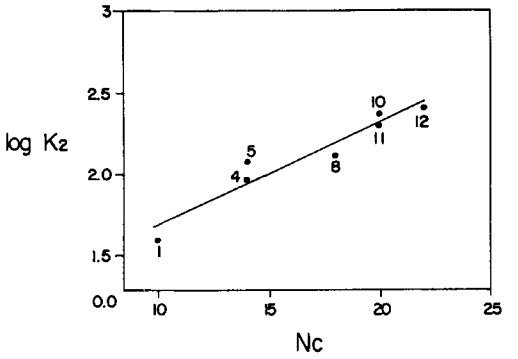


Figure 11: Correlation between binding constants and number of carbons. PAH's identified in Figure 2.

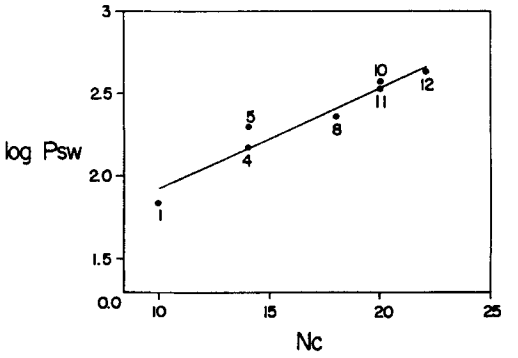


Figure 12: Correlation between distribution coefficients and number of carbons. PAH's identified in Figure 2.

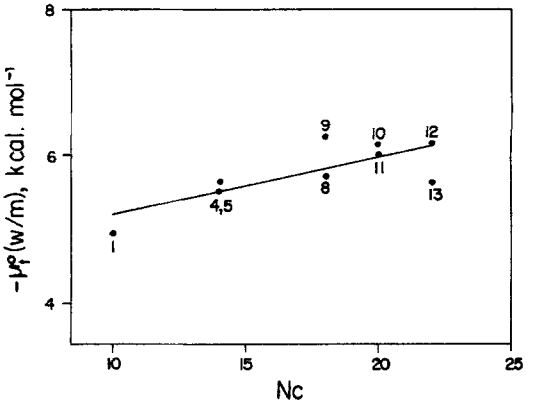


Figure 13: Correlation between transfer energy from water to micelle and the number of carbons. PAH's identified in Figure 2.

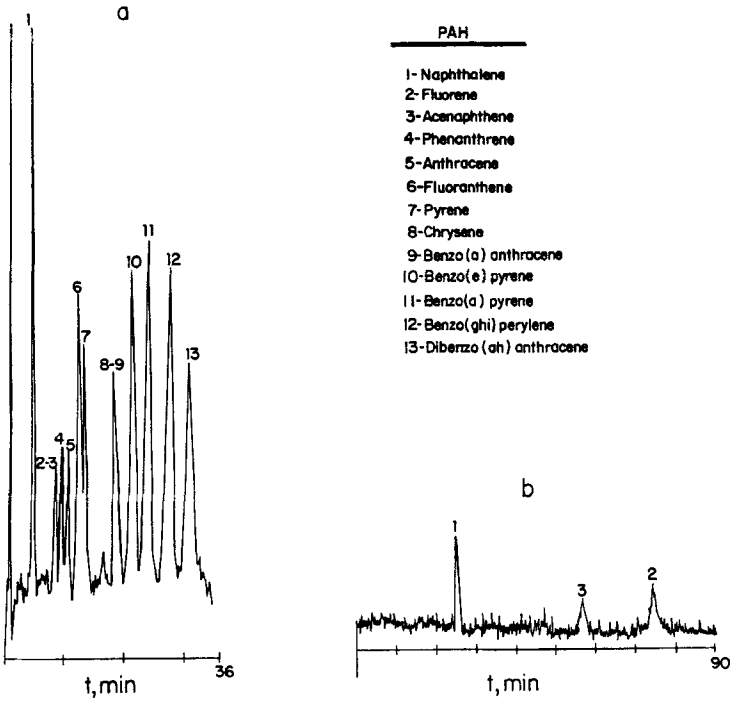
TABLE 3

Optimum experimental conditions

Variable studied	Range	Recommended
Mobile phase micellar concentration	4.10^{-4} - 10^{-1} (M)	5.10^{-2} (M)
Organic modifiers	propanol: 2 - 30% methanol: 20 - 80%	methanol: 50%
Flow-rate	0.4 - 1 (ml/min)	1 (ml/min)
Temperature	30 - 60 (ºC)	60 (ºC)
Stationary phase	C-8 and C-18 columns	C-18

Analytical considerations

The optimum experimental conditions for separating the PAHs studied are summarized in Table 3. In these conditions eleven PAH's can be separated, but only nine PAH's can be resolved to the base-line: naphthalene, fluorene or



Figures 14: Chromatograms of standard PAH's solutions. C-18 column; flow-rate, 1 ml/min; temperature, 60ºC; detection, 254 nm excitation and 375 nm emission (long-pass); mobile phase, 50% methanol/water; a, 0.05 M Brij-35; b, without Brij-35.

acenaphthene, phenanthrene or anthracene, fluoranthene or pyrene, chrysene or benzo(a)anthracene, benzo(e)pyrene, benzo(a)pyrene, benzo(ghi)perylene and dibenzo(ah)anthracene. The chromatogram is shown in Figure 14 a. The chromatogram for 50% methanol/water is also included (Figure 14 b) for comparison; it should be noted that optimum conditions are methanol/water, 85/15 (v/v); up to an analysis time of 90 minutes only naphthalene, acenaphthene and fluorene are

Table 4

Analytical characteristics

PAH	Sensitization factors*	Dynamic range, (μg/ml)	DL**, (ng/ml)	RSD, %***
Naphthalene	1	6.15 - 117	500	2.4
Fluorene	1.1	3.74 - 29.9	250	3.5
Acenaphthene	1.1	3.78 - 432	300	5.5
Phenanthrene	2.7	0.050 - 0.668	4.01	3.4
Anthracene		0.004 - 0.046	0.250	2.6
Fluoranthene	2.5	0.086 - 1.15	10.0	1.8
Pyrene	3.5	0.035 - 0.400	2.02	4.2
Chrysene	1.3	0.093 - 0.748	3.50	2.4
Benzo(a)anthracene	1.3	0.041 - 0.478	1.52	1.7
Benzo(e)pyrene	2.5	0.121 - 1.39	6.51	5.6
Benzo(a)pyrene	2.1	0.026 - 0.297	1.01	1.9
Benzo(ghi)perylene	1.9	0.092 - 1.06	3.50	1.3
Dibenzo(ah)anthracene	1.7	0.272 - 3.12	8.50	2.0

* Defined as the MLC/RPLC areas ratio
** DL = 3 S/N
*** n = 3

resolved, with retention times of 22, 57 and 76 minutes, respectively. The sensitivity of PAH's with fluorimetric detection is significantly higher than that obtained by the classical separation using a mobile phase of methanol/water 85/15, v/v. The sensitization factors defined as the area ratio MLC/RPLC are summarized in Table 4.

Other analytical characteristics are included in this table. The detection limits (DL) have been evaluated as three times the signal-noise ratio (3 S/N); the noise was defined as the peak to peak ratio. The precision, expressed as relative standard deviation (RSD), was determined from three replicates at a concentration level in the middle of the dynamic range.

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