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Prediction of retention for substituted and unsubstituted polycyclic aromatic hydrocarbons in micellar liquid chromatography in the presence of organic modifiers

M.A. Rodríguez Delgado, M.J. Sánchez, V. González, F. García Montelongo*

Department of Analytical Chemistry, Food Science and Toxicology, University of La Laguna, 38071 La Laguna, Spain

Abstract

Statistical and factor analysis have been used to establish some general equations relating retention parameters to molecular descriptors of substituted and unsubstituted polycyclic aromatic hydrocarbons. They allow the determination of the retention behaviour of these solutes in micellar liquid chromatography using “hybrid” sodium dodecyl sulphate–water–alcohol micellar mobile phases and a Nova-Pak C₁₈ column.

1. Introduction

In the past years several models have been published in the context of quantitative structure–retention relationships (QSSRs) in order to predict the retention behaviour in liquid and gas chromatography, and even though linear and non-linear models have been proposed and different statistics have been used, the practical significance of a model depends mostly on the coincidence between the calculated and the experimental data. Critical reviews of many of these efforts have been published by Kaliszan [1] and Lochmüller et al. [2].

Numerous solute-related physicochemical characteristic descriptors, such as molecular connectivity (χ) [3–6], octanol–water partition coefficient ($\log P$) [7,8], Van der Waals volume (V_{vw}) [9–11], length-to-breadth ratio of the molecule (L/B) [12–14], dipole moment (μ) [15,16], etc. or combinations of several descriptors

[3,16,17] have been used in QSSRs, but in chromatographic practice a model is preferable if the descriptors have a clear physicochemical meaning.

It has been suggested that an adequate model should include as many descriptors as possible in order to take all types of interactions in the chromatographic system into account [18]. However, a great number of descriptors in the model can complicate the calculation, even with the use of computers, without ameliorating the model [19]. According to Kaliszan [20] observing all the statistical rules and recommendations, one must select the minimum number of descriptors to produce an equation with good predictive ability.

Polycyclic aromatic hydrocarbons (PAHs) are a unique group of compounds whose mutagenic and carcinogenic properties makes it necessary to develop accurate methods for their separation and identification. Reversed-phase high-performance liquid chromatography (RP-HPLC), with UV and/or fluorescence detection and acetonitrile–water or methanol–water as mobile phases,

* Corresponding author.

has become increasingly popular among researchers for analysis of PAHs.

Micellar liquid chromatography (MLC) which uses aqueous surfactant solutions, at concentrations above the critical micelle concentration, as mobile phases in RP-HPLC, can be considered as an alternative to classical partition chromatography with hydro-organic phases. Some advantages of the technique are the low cost and the non-flammability, non-toxicity and easy disposal of the solvent. However, the low efficiency of these mobile phases has been well established mainly due to the slow mass transfer [21], but raising temperature and adding small amounts of alcohols, giving rise to the so-called "hybrid" mobile phases, enhances the efficiency of the separation and improves retention control [22]. The presence of the alcohol modifier in the system can alter the configuration of the stationary phase, and its incorporation in the micelle can result in additional interaction with solute.

In this paper multiple linear regression models are established between MLC retention parameters, using an anionic micellar system, in the presence of methanol, 2-propanol and *n*-butanol as the modifier, and several molecular descriptors of PAHs.

2. Experimental

2.1. Apparatus

All measurements were made with a Waters 600 multisolvent delivery system equipped with an U6K sample injector, a Waters Lambda-Max 481 LC variable-wavelength spectrophotometric detector operating at 254 nm, and a Baseline Workstation 810. The analytical column was a Waters (Milford, MA, USA) Nova-Pak C₁₈, 150 × 3.9 mm I.D., 4 μm particle diameter. A silica precolumn was employed to saturate the mobile phase with silicate to protect the analytical column and to avoid hydrolysis of the bonded stationary phase. The analytical column and the mobile phase reservoir were water-jac-

keted and temperature-controlled with a circulating bath.

2.2. Reagents

The surfactant sodium dodecyl sulfate (SDS) of electrophoresis grade was obtained from Aldrich (Milwaukee, WI, USA) and used as received. Methanol (MeOH), ethanol, 2-propanol (PrOH) and *n*-butanol (BuOH) were Merck (Darmstadt, Germany) analytical-reagent grade products. Naphthalene (1), acenaphthylene (2), fluorene (3), anthracene (4), phenanthrene (5), fluoranthene (6), pyrene (7), chrysene (8), benz[*a*]anthracene (9), benzo[*b*]fluoranthene (10), benzo[*a*]pyrene (11), benzo[*e*]pyrene (12), perylene (13), dibenz[*a,c*]anthracene (14), dibenz[*a,h*]anthracene (15), benzo[*ghi*]perylene (16), 1-methylanthracene (17), 2-methylanthracene (18), 9-methylanthracene (19), 9,10-dimethylanthracene (20), 2-methylphenanthrene (21) and 3,6-dimethylphenanthrene (22) were Aldrich products. Numbers identify the compound in tables.

2.3. Procedure

The appropriate mass of SDS was dissolved in Milli-Q (Millipore, Bedford, MA, USA) water containing the desired alcohol content, and the solution filtered through a 0.45-μm nylon membrane filter (Whatman, Kent, England). The mobile phase was degassed under vacuum in an ultrasonic bath prior to use. Stock solutions of PAHs were prepared in ethanol, and diluted with the same solvent when necessary.

The void volume of the system was measured by multiple injections of water or sodium nitrate solution and found to be 0.81 ml, and was used for all *k'* calculations. The *k'* values were measured at 60 ± 0.1°C, injections of 20 μl were made. All measurements were made at least five times. Calculated log *k'* are shown in Table 1.

2.4. Descriptors generation

The descriptors considered in this paper were

Table 1
Log k' values for the PAHs studied

PAH	Log k'														
	Methanol (%)					2-Propanol (%)					<i>n</i> -Butanol (%)				
	3	5	10	15	20	3	5	10	15	20	2	4	6	8	10
Naphthalene	1.322	1.314	1.272	1.229	1.183	1.252	1.208	1.113	1.022	0.936	1.202	1.115	1.030	0.959	0.875
Acenaphthylene	1.362	1.351	1.312	1.272	1.231	1.287	1.246	1.147	1.056	0.970	1.238	1.147	1.059	0.985	0.898
Fluorene	1.484	1.471	1.436	1.396	1.358	1.405	1.360	1.265	1.177	1.098	1.346	1.249	1.158	1.081	0.991
Anthracene	1.520	1.508	1.470	1.434	1.398	1.440	1.394	1.295	1.209	1.130	1.380	1.279	1.185	1.106	1.018
Phenanthrene	1.498	1.491	1.450	1.415	1.370	1.417	1.369	1.273	1.182	1.117	1.358	1.263	1.173	1.089	0.998
Fluoranthene	1.565	1.556	1.516	1.481	1.437	1.477	1.425	1.348	1.235	1.171	1.413	1.311	1.219	1.132	1.039
Pyrene	1.574	1.560	1.523	1.484	1.449	1.481	1.434	1.331	1.246	1.171	1.420	1.312	1.218	1.138	1.049
Chrysene	1.679	1.669	1.623	1.590	1.548	1.584	1.527	1.429	1.340	1.277	1.509	1.401	1.305	1.215	1.120
Benz[<i>a</i>]anthracene	1.685	1.670	1.633	1.591	1.557	1.588	1.536	1.433	1.347	1.275	1.516	1.399	1.299	1.214	1.122
Benzo[<i>b</i>]fluoranthene	1.742	1.733	1.689	1.654	1.611	1.637	1.580	1.477	1.388	1.329	1.564	1.444	1.346	1.256	1.158
Benzo[<i>a</i>]pyrene	1.749	1.733	1.696	1.653	1.621	1.645	1.590	1.485	1.402	1.332	1.567	1.445	1.346	1.263	1.171
Benzo[<i>e</i>]pyrene	1.706	1.692	1.648	1.614	1.580	1.609	1.553	1.445	1.358	1.288	1.531	1.411	1.310	1.227	1.136
Perylene	1.711	1.699	1.656	1.616	1.581	1.610	1.554	1.446	1.363	1.293	1.534	1.411	1.312	1.232	1.139
Dibenz[<i>a,c</i>]anthracene	1.815	1.807	1.760	1.721	1.677	1.703	1.640	1.537	1.444	1.383	1.612	1.491	1.387	1.294	1.192
Dibenz[<i>a,h</i>]anthracene	1.796	1.776	1.728	1.690	1.658	1.679	1.624	1.512	1.428	1.360	1.602	1.469	1.363	1.284	1.197
Benzo[<i>ghi</i>]perylene	1.854	1.837	1.793	1.759	1.723	1.746	1.691	1.582	1.497	1.426	1.654	1.526	1.419	1.330	1.235
1-Methylanthracene	1.620	1.606	1.565	1.526	1.493	1.534	1.484	1.382	1.293	1.218	1.467	1.354	1.253	1.168	1.075
2-Methylanthracene	1.641	1.626	1.586	1.550	1.518	1.554	1.503	1.410	1.320	1.248	1.485	1.373	1.275	1.191	1.100
9-Methylanthracene	1.683	1.669	1.629	1.591	1.560	1.595	1.544	1.440	1.350	1.278	1.525	1.407	1.301	1.213	1.117
9,10-Dimethylanthracene	1.616	1.601	1.563	1.523	1.491	1.532	1.478	1.387	1.294	1.219	1.463	1.354	1.255	1.171	1.081
2-Methylphenanthrene	1.707	1.691	1.648	1.609	1.576	1.615	1.559	1.457	1.363	1.289	1.538	1.415	1.307	1.217	1.120
3,6-Dimethylphenanthrene	1.605	1.598	1.556	1.516	1.482	1.518	1.468	1.368	1.281	1.206	1.451	1.342	1.244	1.161	1.069

Standard deviation ≤ 0.0012 .

obtained from the bibliography $\{\log P =$ logarithm of the octanol–water partition coefficient [3], $\chi =$ molecular connectivity index [12], L/B : the ratio of the maximalized length-to-breadth [12], $F =$ correlation factor, calculated for each PAH as (number of double bonds) + (number of primary and secondary carbon atoms) $- 0.5$ for a non-aromatic ring) or calculated using the PC Model V4.0 approach (Serena Software, Bloomington, IN, USA). This program was used to determine the three-dimensional structure of compounds based on energy minimization. After setting up the geometries of the molecules having the smallest minimized-energy, the stretching energy (STR), bond energy (BND), stretch bend constants (SB), torsional energy (TOR), Van der Waals volume (V_{vw}), dipole moment (DPMON), heat of forma-

tion (HF), fraction of non-polar surface area (NSSA/TSA), fraction of non-polar unsaturated surface area (NUSA/TSA) are calculated by the program. These data are shown in Table 2.

3. Results and discussion

In a previous work [19] equations such as $\log k' = f(F, L/B)$ were established to explain the behaviour of unsubstituted PAHs when using SDS micellar mobile phases.

These kinds of equations can also be developed for unsubstituted PAHs when alcohol-modified SDS mobile phases are used and regression coefficients are even better in the presence of these modifiers (Table 3). Furthermore, while the relevance of the hydrophobicity of the

Table 2
Descriptors

PAH	<i>F</i>	<i>L/B</i>	Log <i>P</i>	χ	STR	BND	SB	TOR	V_{ow}	DPMON	HF	NSSA/ TSA	NUSA/ TSA
Naphthalene	5.0	1.24	3.35	3.408	0.17	0.07	0.00	12.00	5.39	0.00	35.00	0.6091	0.3972
Acenaphthylene	5.5	1.08	3.82	4.149	0.68	14.40	0.07	17.00	3.83	0.90	64.88	0.5629	0.4391
Fluorene	6.5	1.57	3.99	4.612	0.27	9.06	-0.08	13.70	3.76	0.32	48.22	0.6278	0.3734
Anthracene	7.0	1.57	4.63	4.809	0.28	0.15	0.01	18.00	8.14	0.01	55.79	0.5845	0.4195
Phenanthrene	7.0	1.46	4.63	4.815	0.47	0.68	0.03	19.00	9.34	0.16	49.35	0.5776	0.4224
Fluoranthene	8.0	1.22	5.22	5.565	0.59	15.29	0.02	25.00	6.02	0.44	70.69	0.5508	0.4519
Pyrene	8.0	1.27	5.22	5.559	0.32	0.19	0.01	24.00	9.74	0.00	57.59	0.5707	0.4298
Chrysene	9.0	1.72	5.91	6.226	0.85	1.41	0.07	26.00	13.70	0.00	66.15	0.5540	0.4485
Benz[<i>a</i>]anthracene	9.0	1.58	5.92	6.220	0.58	0.80	0.04	25.00	12.20	0.24	68.44	0.5694	0.4335
Benzo[<i>b</i>]fluoranthene	10.0	1.40	6.62	6.976	0.79	15.07	0.02	32.00	9.40	0.61	83.39	0.5404	0.4609
Benzo[<i>a</i>]pyrene	10.0	1.50	6.50	6.970	0.62	0.79	0.04	31.00	13.84	0.15	76.03	0.5571	0.4435
Benzo[<i>e</i>]pyrene	10.0	1.12	6.50	6.976	0.96	1.42	0.07	32.00	15.80	0.19	73.00	0.5401	0.4633
Perylene	10.0	1.27	6.58	6.976	0.96	1.52	0.06	32.00	15.71	0.00	79.94	0.5292	0.4750
Dibenz[<i>a,c</i>]anthracene	11.0	1.24	7.19	7.637	1.37	2.42	0.12	33.00	19.35	0.10	85.85	0.5504	0.4500
Dibenz[<i>a,h</i>]anthracene	11.0	1.12	6.85	7.720	0.82	1.43	0.06	32.00	16.19	0.00	81.58	0.5471	0.4546
Benzo[<i>ghi</i>]perylene	11.0	1.79	7.18	7.631	0.71	0.76	0.03	37.00	15.63	0.39	77.33	0.5372	0.4641
1-Methylanthracene	8.0	1.41	5.14	5.226	0.42	0.43	0.03	17.01	9.04	0.17	49.43	0.6306	0.3730
2-Methylanthracene	8.0	1.74	5.14	5.220	0.32	0.25	0.02	17.28	8.48	0.35	48.11	0.6398	0.3602
9-Methylanthracene	9.0	1.23	5.65	5.655	0.71	1.41	0.07	16.74	10.71	0.18	53.59	0.6317	0.3725
9,10-Dimethylanthracene	8.0	1.58	5.14	5.226	0.89	1.60	0.08	17.66	12.36	0.01	51.51	0.6814	0.3191
2-Methylphenanthrene	9.0	1.26	5.65	5.637	0.49	0.81	0.04	18.28	9.64	0.34	41.52	0.6291	0.3719
3,6-Dimethylphenanthrene	8.0	1.38	5.14	5.232	0.55	0.91	0.05	17.56	9.98	0.40	33.37	0.6911	0.3131

molecule remains almost constant (*a* coefficient), the shape (*b* coefficient) increases its relevance as methanol and propanol concentrations increase, when butanol is the modifier the relevance of both factors decreases on increasing its concentration which can be related to its hydrophobicity.

However, when log *k'* values for the methyl-substituted PAHs are included in these equations, the corresponding regression coefficients decrease in both normal and "hybrid" mobile phases. Moreover, when using, i.e., the equation $\log k' = f(c, F, L/B)$ with hybrid mobile phases two straight lines can be observed on comparing calculated and experimental values (Fig. 1), one of them enclosing the unsubstituted PAHs ($R^2 = 0.997$) and the other enclosing the methyl-substituted PAHs ($R^2 = 0.926$), if the whole set of data are regressed all together the regression coefficient decreases even more ($R^2 = 0.985$). This effect has already been reported in RP-HPLC [23,24] and related to the non-planarity of the methyl-PAHs due to the presence of the methyl

group in the so-called "bay-region" of the PAH structure.

Thus, it is necessary to establish a new model which can explain the behaviour of both unsubstituted and methyl-substituted PAHs. In this way the thirteen starting descriptors in Table 2 were subjected to factor analysis and the results are shown in Table 4. If the number of factors is chosen with the ordinary rule of selecting the ≥ 1 "eigenvalues", then four factors (*F*) are extracted, which account for the 93% of the total variance; thus only four factors were selected for further calculations.

Taking into consideration the factor loadings for the thirteen descriptors in the varimax rotated factor matrix (Table 4), one can see that descriptors *F* and NUSA/TSA explain 96% of the variance on F_1 and F_2 , respectively, DPMON explains 92% of the variance of F_3 and *L/B* the 84% of F_4 . That is, the retention behaviour of PAHs in this hybrid micellar mobile phase can be explained as a function of these four descriptors namely: *F* and *L/B*, geometric descriptors whose

Table 3
Regression equation for the retention of unsubstituted PAHs with a 0.15 M SDS mobile phase containing several concentration of alcohols

Statistical parameter	$\text{Log } k' = aF + bL \cdot B + c^a$										
	Fraction of alcohol in the mobile phase (% v/v)										
	2	3	4	5	6	8	10	15	20	25	30
<i>Methanol</i>											
<i>a</i>		0.0791		0.0782			0.0770	0.0775	0.0786	0.0830	0.0880
<i>b</i>		0.0892		0.0887			0.0934	0.0964	0.0972	0.1020	0.1058
<i>c</i>		0.8231		0.8195			0.7816	0.7367	0.6867	0.6296	0.5662
R^2		0.997		0.997			0.996	0.997	0.996	0.995	0.995
S.D.		0.0083		0.0084			0.0094	0.0091	0.0096	0.0119	0.0124
<i>F</i>		2725.6		2650.5			2026.3	2197.5	2059.5	1483.5	1523.8
<i>2-Propanol</i>											
<i>a</i>		0.0721		0.0695			0.0669	0.0678	0.0708	0.0758	0.0808
<i>b</i>		0.0940		0.0941			0.0956	0.1022	0.1047	0.1059	0.1077
<i>c</i>		0.7825		0.7538			0.6729	0.5672	0.4671	0.3391	0.2125
R^2		0.998		0.997			0.994	0.997	0.997	0.997	0.997
S.D.		0.0071		0.0081			0.0105	0.0080	0.0083	0.0077	0.0083
<i>F</i>		3162.0		2251.3			1257.0	2217.6	2234.7	2958.4	2910.5
<i>n-Butanol</i>											
<i>a</i>	0.0662		0.0592		0.0561	0.0540	0.0526				
<i>b</i>	0.0842		0.0866		0.0850	0.0769	0.0722				
<i>c</i>	0.7780		0.7241		0.6569	0.6027	0.5296				
R^2	0.997		0.996		0.996	0.997	0.997				
S.D.	0.0074		0.0072		0.0076	0.0056	0.0062				
<i>F</i>	2462.9		2059.8		1681.1	2858.5	2219.9				

^a $R^2 = 0.990$ when no alcohol modifier is present [19].

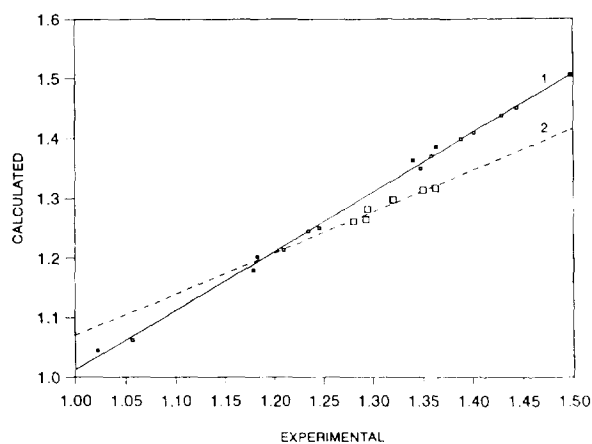


Fig. 1. Plot of calculated vs. experimental values according to the equation $\log k' = f(F, L, B)$, with a 0.15 M SDS and 15% 2-propanol in water mobile phase. 1 = Unsubstituted PAHs; 2 = methyl-substituted PAHs.

relevance in the factor analysis reveals the importance of the size and shape of the molecule; NUSA/TSA, giving information about the electron delocalization along the aromatic rings, and DPMON, an electronic descriptor giving information about the dispersive forces. The relevance of these latter descriptors is in agreement with the idea that polarity can play an important role in the retention behaviour of PAHs. Although the total dipole moment has occasionally been reported as a significant parameter in QSRR equations, it has been shown that it can not be used to describe all specific polar features of the compounds [25]. However, one can see in Table 2 how DPMON acquires importance in describing the electronic properties of isomers (i.e., 1-methylantracene 0.17 D, 2-methylantracene 0.35 D).

Table 4
Factor analysis of the thirteen molecular descriptors

Eigenvalue	7.141	2.486	1.469	0.916	0.570				
% variance	54.9	74.1	85.4	92.4	96.8				
Variable	Unrotated matrix				Estimated communality	Varimax rotated			
	F_1	F_2	F_3	F_4		F_1	F_2	F_3	F_4
Log P	0.93849	0.19255	-0.03566	0.25065	0.98194	0.95240	0.25381	-0.09965	-0.02276
χ	0.95486	0.09643	0.02802	0.23145	0.97541	0.91864	0.35596	-0.05505	-0.04199
L/B	-0.09302	0.37045	0.62115	0.49676	0.77848	0.07518	-0.10550	-0.22455	-0.84337
F	0.90699	0.23686	-0.05548	0.29255	0.96740	0.95901	0.18660	-0.10396	-0.04563
STR	0.82766	0.00809	-0.44345	-0.03855	0.88322	0.78825	0.18964	0.03632	0.47392
BND	-0.05362	-0.88977	-0.17547	0.27000	0.89825	-0.12448	0.24647	0.90374	0.07256
SB	0.59479	0.23867	-0.57807	-0.21859	0.79268	0.60937	-0.05366	-0.17477	0.62284
TOR	0.95970	-0.14575	0.13001	0.03257	0.96024	0.75512	0.62298	0.00164	0.04388
V_{vw}	0.88862	0.43646	-0.05847	-0.07392	0.98903	0.84636	0.21472	-0.44805	0.16079
DPMON	-0.16525	-0.73507	-0.38094	0.39862	0.87165	-0.07825	-0.05621	0.92270	0.10480
HF	0.89256	-0.36217	0.13504	0.01360	0.94625	0.63353	0.71518	0.16787	0.07237
NSSA/TSA	-0.68906	0.50732	-0.40844	0.29187	0.98419	-0.23582	-0.96027	-0.05825	-0.05533
NUSA/TSA	0.68276	-0.50696	0.40794	-0.30712	0.98391	0.22450	0.96269	0.05085	0.06448

In order to check the latter conclusion, regression analysis was carried out on $\log k'$, F , L/B , NUSA/TSA and DPMON and the resulting equations are shown in Table 5. As can be seen, for a 0.15 M SDS mobile phase and for each percentage of alcohol modifier, the equation obtained shows a high correlation coefficient, a low standard deviation and a high Fisher F value, as well as a small number of independent variables [20].

Furthermore, from these equations it can be deduced that the retention of a PAH in MLC increases as: (1) F and L/B , size and shape of the molecule increase; we must remember that hydrophobicity increases with the size of the molecule as F and $\log P$ are highly correlated ($R^2 = 0.993$, $n = 22$) and appear on F_1 with high factor loadings; (2) as the dipole moment DPMOM increases, however, the loading of this descriptor is small when compared with F and L/B and serves mainly to differentiate between methyl-substituted isomers; and (3) as the NUSA/TSA ratio decreases, this descriptor gives information about the electron delocalization on the aromatic rings, thus retention in-

creases as electrons are more localized. This effect can be clearly seen taking anthracene, 2-methylanthracene and 9,10-dimethylanthracene, which elute in this order, as an example. In their electronic maps (Fig. 2), one can see that the presence of a methyl substituent increases the electron localization (δ^-), this effect being more relevant for 9,10-dimethylanthracene, due to the inductive effect (δ^+) of the methyl group.

The results in Table 5 show that the coefficients of the independent variables change with the alcohol modifier and its percentage in the mobile phase (Fig. 3). In general, as the alcohol hydrophobicity increases the solute hydrophobicity becomes less important, due to competitiveness of both alcohol and solute for the micelles. The same effect is observed as the alcohol percentage increases, until ca. 20% for methanol and propanol and ca. 6% for n -butanol, which can be related to the micelles losing their integrity at the higher modifier percentages [26,27] and to the formation of mixed micelles [28,29].

Given that the coefficients for the independent

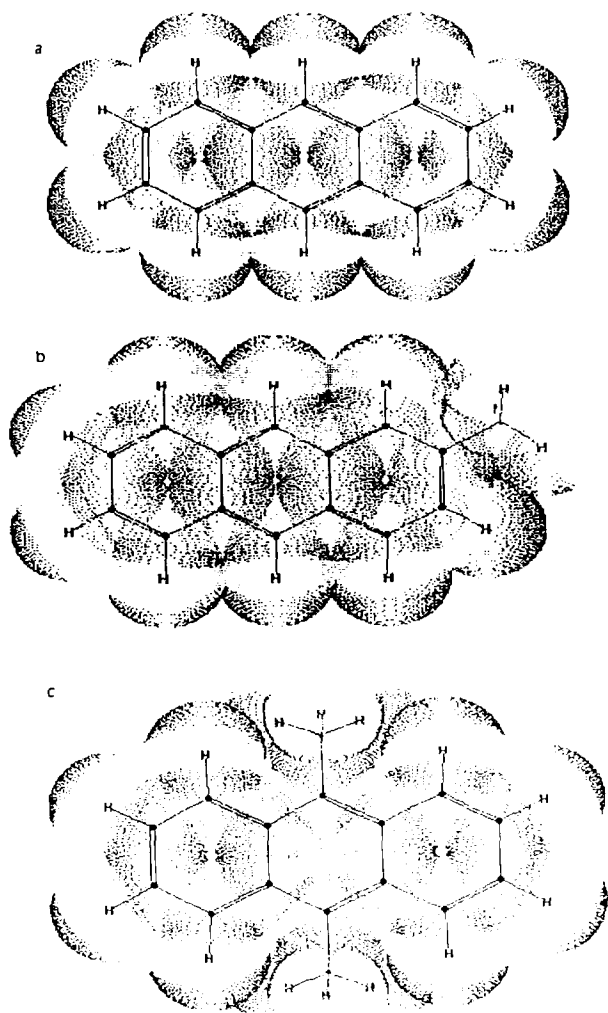


Fig. 2. The three-dimensional structure of (a) anthracene, (b) 2-methylanthracene and (c) 9,10-dimethylanthracene obtained by molecular modeling. The darker dots indicate the polar surface region on the molecules.

variables change as the percentage of the alcohol modifier in the mobile phase changes, it will be possible to develop a more general equation where this percentage will be taken into account, and valid up to ca. 20% methanol or 2-propanol or ca. 6% *n*-butanol in the mobile phase. The results are shown in Table 6.

Furthermore, as previously shown [30] the best selectivity for this group of PAHs on a C_{18} column is obtained with a 15% 2-propanol and ca. 0.15 *M* SDS in water mobile phase; an

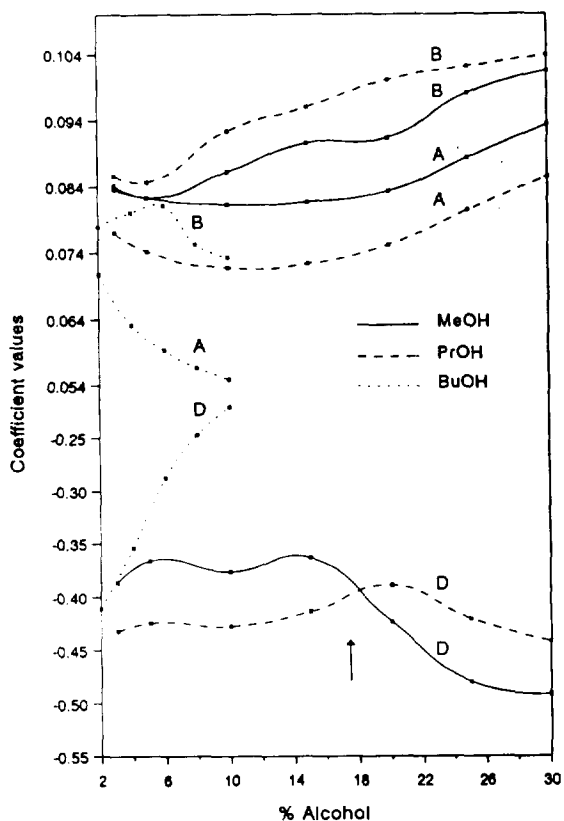


Fig. 3. Plot of the coefficients of the independent variables in the equations in Table 5 as a function of the alcohol percentage.

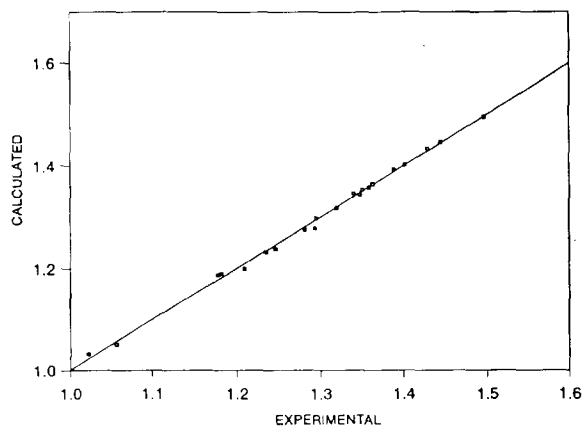


Fig. 4. Plot of calculated vs. experimental values according to Eq. b in Table 6, for a 0.15 *M* SDS and 15% 2-propanol in water mobile phase.

Table 5

Regression equation for the prediction of the retention of substituted and unsubstituted PAHs using a 0.15 M SDS mobile phase in the presence of alcohol modifier

Statistical parameter	Log $k' = aF + bL/B + cDPMON + dNUSA/TSA + e$										
	Fraction of alcohol in the mobile phase (% v/v)										
	2	3	4	5	6	8	10	15	20	25	30
<i>Methanol</i>											
<i>a</i>		0.08349		0.08232			0.08130	0.08165	0.08328	0.08825	0.09328
<i>b</i>		0.08412		0.08239			0.08623	0.09057	0.09134	0.09809	0.10148
<i>c</i>		0.02076		0.01868			0.01874	0.02080	0.02067	0.02577	0.02355
<i>d</i>		-0.38637		-0.36638			-0.37651	-0.36369	-0.42398	-0.47994	-0.49158
<i>e</i>		0.95818		0.95027			0.91665	0.86354	0.83691	0.79629	0.73895
R^2		0.998		0.997			0.997	0.996	0.996	0.993	0.993
S.D.		0.00716		0.00768			0.00784	0.00805	0.00858	0.01178	0.01246
<i>F</i>		1896.7		1610.5			1506.9	1445.7	1313.4	776.4	779.2
<i>2-Propanol</i>											
<i>a</i>		0.07696		0.07419			0.07174	0.07229	0.07509	0.08033	0.08546
<i>b</i>		0.08555		0.08472			0.09236	0.09605	0.10016	0.10212	0.10384
<i>c</i>		0.01773		0.01690			0.02422	0.01727	0.01957	0.01901	0.01726
<i>d</i>		-0.43211		-0.42466			-0.42786	-0.41415	-0.38960	-0.42126	-0.44240
<i>e</i>		0.93966		0.90970			0.81941	0.71596	0.60378	0.48718	0.36937
R^2		0.997		0.996			0.994	0.996	0.997	0.997	0.997
S.D.		0.00717		0.00783			0.00929	0.00710	0.00755	0.00728	0.00794
<i>F</i>		1596.3		1246.6			825.5	1452.5	1394.2	1709.1	1625.9
<i>n-Butanol</i>											
<i>a</i>	0.07077		0.06315		0.05933	0.05672	0.05493				
<i>b</i>	0.07791		0.08008		0.08106	0.07525	0.07328				
<i>c</i>	0.02017		0.01661		0.01636	0.01270	0.0112				
<i>d</i>	-0.41099		-0.35420		-0.28759	-0.24710	-0.22116				
<i>e</i>	0.92414		0.85165		0.75760	0.68840	0.60393				
R^2	0.996		0.995		0.995	0.997	0.996				
S.D.	0.00721		0.00696		0.00683	0.00499	0.00563				
<i>F</i>	1329.2		1148.4		1067.6	1843.7	1366.2				

equation has been developed for this mobile phase (Table 6). Obviously a more general equation could be established as a simultaneous function of X , percentage of propanol, and C , concentration of surfactant, in the mobile phase which allows the prediction of the retention for this group of PAHs for any given chromatographic conditions using such a mobile phase. However, much more experimental work would be necessary for this objective.

The calculated $\log k'$ values according to Eq. b in Table 6, are plotted in Fig. 4 against the experimental values for the twenty two unsubstituted and methyl-substituted PAHs. The equa-

tion of the fitted straight line (linear least squares) was: $\log k'_{\text{calc}} = 0.0038 + 0.997 \log k'_{\text{exp}}$ ($R^2 = 0.997$, standard error of estimate = 0.00653). The high correlation coefficient, the proximity of the slope to unity and the low intercept revealed the absence of systematic errors.

4. Conclusions

Equations previously developed for the prediction of the retention of unsubstituted PAHs in micellar SDS mobile phases, can be also used for

Table 6
General regression equation for the prediction of the retention of substituted and unsubstituted PAHs using X (%) alcohol and C (M) SDS in water mobile phase

$$\text{Log } k' = a(X)F + b(X)L/B + c(X)\text{DPMON} + d(X)\text{NUSA/TSA} + e(X)$$

Eq. a: methanol ($\leq 20\%$)

$$\begin{aligned} \text{Log } k' = & (8.57 \cdot 10^{-2} - 9.15 \cdot 10^{-4}X + 5.35 \cdot 10^{-5}X^2 - 6.94 \cdot 10^{-7}X^3)F \\ & + (8.89 \cdot 10^{-2} - 2.53 \cdot 10^{-3}X + 3.12 \cdot 10^{-4}X^2 - 8.96 \cdot 10^{-6}X^3)L/B \\ & + (2.64 \cdot 10^{-2} - 2.59 \cdot 10^{-3}X + 2.46 \cdot 10^{-4}X^2 - 6.58 \cdot 10^{-6}X^3)\text{DPMON} \\ & + (4.29 \cdot 10^{-1} + 1.66 \cdot 10^{-2}X - 8.20 \cdot 10^{-4}X^2)\text{NUSA/TSA} \\ & + (9.45 \cdot 10^{-1} + 8.34 \cdot 10^{-3}X - 1.59 \cdot 10^{-3}X^2 + 4.50 \cdot 10^{-5}X^3) \end{aligned}$$

Eq. b: 2-propanol ($\leq 20\%$)

$$\begin{aligned} \text{Log } k' = & (8.24 \cdot 10^{-2} - 2.25 \cdot 10^{-3}X + 1.39 \cdot 10^{-4}X^2 - 2.24 \cdot 10^{-6}X^3)F \\ & + (8.26 \cdot 10^{-2} + 1.04 \cdot 10^{-3}X - 8.18 \cdot 10^{-6}X^2)L/B \\ & + (1.71 \cdot 10^{-2} - 5.77 \cdot 10^{-4}X + 3.00 \cdot 10^{-5}X^2)\text{DPMON} \\ & + (-4.28 \cdot 10^{-1} - 1.97 \cdot 10^{-3}X + 1.94 \cdot 10^{-4}X^2)\text{NUSA/TSA} \\ & + (9.86 \cdot 10^{-1} - 1.42 \cdot 10^{-2}X - 2.44 \cdot 10^{-4}X^2) \end{aligned}$$

Eq. c: *n*-butanol ($\leq 6\%$)

$$\begin{aligned} \text{Log } k' = & (8.30 \cdot 10^{-2} - 7.58 \cdot 10^{-3}X + 7.87 \cdot 10^{-4}X^2 - 3.09 \cdot 10^{-5}X^3)F \\ & + (7.23 \cdot 10^{-2} + 3.34 \cdot 10^{-3}X - 3.24 \cdot 10^{-4}X^2)L/B \\ & + (2.40 \cdot 10^{-2} - 2.14 \cdot 10^{-3}X + 8.77 \cdot 10^{-5}X^2)\text{DPMON} \\ & + (-4.60 \cdot 10^{-1} + 1.92 \cdot 10^{-2}X + 3.00 \cdot 10^{-3}X^2 - 2.54 \cdot 10^{-4}X^3)\text{NUSA/TSA} + (1.01 - 4.02 \cdot 10^{-2}X) \end{aligned}$$

$$\text{Log } k' = a(C)F + b(C)L/B + c(C)\text{DPMON} + d(C)\text{NUSA/TSA} + e(C)$$

Eq. d: SDS (15% 2-propanol)

$$\begin{aligned} \text{Log } k' = & (1.46 \cdot 10^{-1} - 1.230C + 7.88C^2 - 19.60C^3)F \\ & + (1.77 \cdot 10^{-1} - 1.118C + 3.86C^2)L/B \\ & + (5.70 \cdot 10^{-2} - 5.66 \cdot 10^{-3}C + 2.027C^2)\text{DPMON} \\ & + (-1.72 \cdot 10^{-1} - 6.547C + 32.86C^2)\text{NUSA/TSA} \\ & + (1.209 - 6.263C + 41.00C^2 - 140.52C^3) \end{aligned}$$

X = Volume fraction of alcohol modifier, at 0.15 M SDS; C = concentration of SDS in presence of 2-propanol.

“hybrid” alcohol–SDS–water mobile phases with even better regression coefficients, showing that in both cases retention is mainly a function of size and shape of the molecules. However the prediction is no longer valid when dealing with methyl-substituted PAHs.

In the new equations developed for both unsubstituted and methyl-substituted PAHs, electronic parameters are included to take into account the electron distribution in the molecules. The coefficient of the independent variables in these equations show a quite different behaviour of the hybrid mobile phases containing methanol or 2-propanol from those contain-

ing butanol, which is related to their different hydrophobicity.

Furthermore, for ca. 20% methanol or propanol and ca. 6% butanol a changing behaviour is also observed and related to the micelles losing their integrity and to the formation of mixed micelles.

The equations developed to model the retention behaviour of both unsubstituted and methyl-substituted PAHs are useful for the optimization of the resolution in their separation using the elution data for a reduced number of compounds. However, more work is necessary on the optimum prediction equation.

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