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New insights on the retention mechanism of non-polar solutes in reversed-phase liquid chromatographic columns

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Abstract

A clarification of the retention mechanism of non-polar solutes in octadecyl reversed-phase chromatographic columns is attempted based on a systematic comparison of the retention in C_{18} and C_2 columns under the assumption that the retention in C_2 columns is due to adsorption. The comparison involves curve fitting procedures and tests based on the properties of special functions suggested in the present paper. For the application of this approach the retention behaviour of six non-polar solutes, benzene, toluene, ethylbenzene, propylbenzene, isopropylbenzene and *tert*-butylbenzene, is studied from aqueous mobile phases modified with methanol, isopropanol, acetonitrile and tetrahydrofuran using C_{18} and C_2 reversed-phase columns. It was found that the retention mechanism in C_{18} columns is not the same in the four modifiers. In particular, our results show that the adsorption mechanism has a significant contribution in mobile phases modified by acetonitrile and tetrahydrofuran, the partition mechanism is likely to predominate in isopropanol–water mobile phases provided that the mole fraction of isopropanol is higher than 0.2, whereas the case of MeOH is rather obscure, since the various tests did not give a clear picture about the retention mechanism in methanol–water mobile phases.

Keywords: Retention mechanisms; Mobile phase composition; Benzene; Toluene; Alkylbenzenes

1. Introduction

The elucidation of the retention mechanism in reversed-phase liquid chromatographic columns has triggered intensive studies during the last two decades and up to now two principal mechanisms have been proposed [1–17]: *adsorption* of all constituents of the mobile phase, including the solute, on the tips or/and the stem of the hydrocarbon chains of the stationary phase and *partition* of the solute only molecules between the mobile phase and cavities formed within the hydrocarbon chains. In a recent study we have shown that the dominant retention mechanism in reversed-phase C_{18} chromatographic columns is due to adsorption, whereas the partition is likely only for solutes with small and non-polar molecules [1].

In the present study we examine in more detail the retention of small and non-polar solutes using two reversed-phase columns, C_{18} and C_2. In C_2 columns the partition mechanism should be excluded due to the short length (two carbon atoms) of the carbon chains of the stationary phase. Thus the comparison of the chromatographic behaviour in these two types of columns is expected to shed more light in the retention mechanism.

2. Theory

As discussed above, we consider that two principal mechanisms determine the retention in reversed-phase liquid chromatography (RPLC): *adsorption* and *partition*. These two mechanisms may act simultaneously or one may prevail over the other. Thus in C_2 columns the partition mechanism should be reasonably excluded, because there is no room in the stationary phase for the solute molecules. Other phenomena, like the dependence of the conformation of the hydrocarbon chains upon the composition of the mobile phase [2,8,9], the collapse of the chains at water rich mobile phases [2,8,9,18,19], steric effects or charge exclusion [20] may significantly affect the retention of a solute in reversed-phase columns. For this reason these phenomena are taken indirectly into account in the interpretation of the results of the present investigation.

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In order to elucidate the retention mechanism in C_{18} columns we adopted the systematic comparison of the retention behaviour of small and non-polar solutes in these columns to that in C_2 columns. This approach involves several tests based on usual curve fitting procedures as well as on the properties of three special functions that are proposed for the first time in this paper. In particular we adopted the following tests:

2.1. Curve fitting procedures

In these tests, the experimental $\ln k$ upon φ data, where k is the retention factor and φ the volume fraction of the modifier in the mobile phase, are fitted to the theoretical equation that arises from the partition mechanism [1]:

$$\ln k = \ln k_0 + \ln f_A^m + Y \tag{1}$$

where

$$Y = \ln \frac{1-\alpha+\alpha x}{(1-\alpha)(1+\delta)} = -\ln(1+\delta-\alpha\varphi) \approx -\ln(1-\alpha\varphi) \tag{2}$$

and

$$\alpha = 1 - \frac{\rho_B/M_B}{\rho_w/M_w} \tag{3}$$

Here, δ is the percentage contraction of the mobile phase volume caused by the mixing of its constituents, x the organic modifier mole fraction, ρ_B, ρ_w the densities of the pure organic modifier and water, respectively, M_B, M_w are their molecular masses, and f_A^m the activity coefficient of the solute in the mobile phase.

Note that the activity coefficients f_A^m appearing in Eq. (1) can be taken from literature for the solutes we examined, since these coefficients have been determined by headspace gas chromatography [21]. In addition, the contraction δ can be easily calculated from density measurements [1]. Thus Eq. (1) has just one adjustable parameter, parameter $\ln k_0$, and therefore a good fit is strong evidence that the partition mechanism is valid. Note also that the adsorption mechanism leads to equations that exhibit a great number of adjustable parameters (the least number of adjustable parameters is four) and therefore serious difficulties in their numerical applications [1]. Consequently, a good fit of the adsorption model may be the result of the great number of adjustable parameters in combination with the very simple shapes of the experimental $\ln k$ versus φ plots. For this reason we have not proceeded to tests of the adsorption model developed in [1].

2.2. Special functions

The special functions we suggest for the elucidation of the retention mechanism in C_{18} columns are the following:

$$F = \ln k - \ln f_A^m - Y \tag{4}$$

$$S = \ln k_{C_{18}} - \ln k_{C_2} \tag{5}$$

and

$$P(\lambda) = \ln(k_{C_{18}} - \lambda k_{C_2}) - \ln f_A^m - Y \tag{6}$$

These functions exhibit the following properties:

(a) From Eq. (1) we readily obtain that

$$F = \ln k_0 \tag{7}$$

Therefore, if the partition mechanism is valid, then
(i) the plots of F versus φ or x for all solutes at a certain modifier should be straight lines parallel to the φ or x-axis, and
(ii) the plots of F versus φ or x for the certain solute at various modifiers should coincide to one straight line parallel to the φ or x-axis.
Note that a test similar to point (i) has been proposed by Cheong and Carr [22].

(b) If the adsorption mechanism is valid in a reversed-phase column, then we have [1]:

$$\ln k = \ln k^0 + \ln \frac{1-\theta}{1-x} + \ln \frac{f_A^m f_w^s}{f_A^s f_w^m} + Y \tag{8}$$

where θ is the surface coverage by the modifier molecules of the carbon chains of the stationary phase, f denotes the activity coefficient, subscripts A and w denote the solute and the solvent (water), respectively, and superscripts m and s denote the mobile phase and the adsorption layer (stationary phase), respectively. The surface coverage θ is determined by the adsorption isotherm, which may be expressed as [1]:

$$\ln \frac{f_B^s \theta}{f_w^s (1-\theta)} = \ln \beta + \ln \frac{f_B^m x}{f_w^m (1-x)} \tag{9}$$

where subscript B denotes the organic modifier and β is an adsorption equilibrium constant.

Here, we shall show that the assumption that the extent of adsorption of a certain modifier is almost the same in the C_{18} and C_2 columns, provided that the mobile phase composition is the same in these two columns, is a very reasonable approximation. It is known that the adsorption of a compound from a polar solvent on a certain adsorbing surface (substrate) is primarily governed by the solubility of this compound in the solvent [23,24]. The less soluble a compound is, the higher its θ value is. The nature of the adsorbing surface affects θ through the substrate–adsorbate interactions. It is evident that the more attractive these interactions are the greater the adsorption extent is. However, for the adsorption of a certain modifier onto the hydrocarbon chains of a C_{18} or C_2 column, the nature of the adsorbing surface is, in fact, the same. The weak, van der Waals type, attractive interactions between the aliphatic chains and an adsorbed molecule of the modifier are expected to be almost the same in the two columns, because the length of the chains is not expected to have any significant effect on these

interactions. Therefore, we can reasonably assume that θ is approximately the same in the two columns.

The same conclusion arises from the adsorption isotherm, Eq. (9). The quantity $\ln\beta$ is the standard free energy of adsorption and therefore its value is determined from the substrate–adsorbate interactions at the adsorbed layer and the various interactions of the modifier in the mobile phase [23]. Thus, for reasons explained in the previous paragraph, $\ln\beta$ is expected to be almost the same in C_{18} and C_2 columns. Note also that the right hand side of Eq. (9) is the same for these two columns. In what concerns the activity coefficients f_B^s, f_w^s appearing in the left hand side of Eq. (9), we may observe the following. The coefficients f_B^s, f_w^s as well as f_A^s may be expressed as $\ln f_j^s = B_{1j} + B_{2j}\theta + B_{3j}\theta^2 + B_{4j}\theta^3$, where B_{1j}, B_{2j}, B_{3j}, B_{4j} depend upon the lateral interactions of the constituents of the adsorbed layer [25]. These interactions depend almost exclusively on the orientation of the adsorbed particles. However, in the case of physical adsorption from solution on a solid substrate, the orientation of the constituents of the adsorbed layer is determined decisively from the interactions of the adsorbed particles with the particles of the solution. The corresponding interactions among the adsorbed particles and the substrate have usually a small effect on the orientation. Thus if we take into account that the nature of the substrate in C_{18} and C_2 columns is almost the same, we readily conclude that $\ln f_j^s = f_j(\theta)$, where the function f_j is independent of the type of the column. Now from Eq. (9) we conclude again that θ should be also approximately independent of the type, C_{18} or C_2, of the column.

From the arguments presented above, we find out that for a certain modifier Eq. (8) may be approximately written as:

$$\ln k_c = \ln k_c^0 + G(x,\theta) \tag{10}$$

where $c = C_{18}$or C_2 and $G(x, \theta)$ is a function of x and θ. Moreover, we have shown that θ is a function of x only through the adsorption isotherm and therefore the quantity at a certain x value is equal to:

$$S = \ln k_{C_{18}} - \ln k_{C_2} = \ln k_{C_{18}}^0 - \ln k_{C_2}^0 = \text{constant} \tag{11}$$

which shows that if the plot of S versus φ or x is a straight line parallel to the x-axis, the adsorption should be the predominant retention mechanism.

Note that the above property of the S function is based on the assumption that the adsorption on the chains of the stationary phase is homogeneous and monolayer. If the modifier forms a polylayer [26], then Eqs. (8) and (9) are no longer valid but Eq. (10) still holds. This conclusion arises from the derivation of Eq. (8) in [1], which readily reveals that the details of the adsorption model determine the precise expression of the function $G(x, \theta)$. Moreover, the adsorption isotherm, irrespective of its details, is a function between x and θ. This means that G is a function of x only and therefore the formation of polylayers is unlikely to affect the validity of Eq. (11). Heterogeneity effects may appear if the adsorbed particles interact not only with the aliphatic chains but also with the silica of the stationary phase. If this happens to a significant extent and the surface heterogeneity is different in the two columns, C_2 and C_{18}, because the surface area of silica interacting with adsorbed molecules or/and the material of silica itself is different in these two columns, then the adsorption isotherm will depend on the column. This means that the function $G(x, \theta)$ will not be the same at a certain x in the two columns and therefore the plot of S versus x may not be a straight line parallel to the x-axis even if the adsorption determines the retention mechanism in C_{18} columns.

(c) In general, we may write:

$$k_{C_{18}} = a k_{C_{18},\text{adsorption}} + (1-a) k_{C_{18},\text{partition}} \quad \text{and}$$
$$k_{C_2} = k_{C_2,\text{adsorption}} \tag{12}$$

where a is the degree of the adsorption contribution to $k_{C_{18}}$. In addition, we have shown above that $\ln k_c = \ln k_c^0 + F(x,\theta)$, $c = C_{18}$ or C_2, which in combination with Eqs. (1) and (12) yields:

$$P(\lambda) = \ln(k_{C_{18}} - \lambda k_{C_2}) - \ln f_A^m - Y$$
$$= \ln(1-a) + \ln k_0 = \text{constant} \tag{13}$$

provided that

$$\lambda = \frac{a k_{C_{18}}^0}{k_{C_2}^0} \tag{14}$$

Consequently, we should always find a value of $\lambda \geq 0$ such that the plot of $P(\lambda)$ versus x becomes parallel to the x-axis. If $\lambda \approx 0$, then the partition predominates in the retention mechanism, otherwise we have a contribution from the adsorption, although the degree of this contribution seems to be indefinable.

At this point we should stress again that the above properties of the functions F, S and $P(\lambda)$ are expected to be strictly valid in the ideal case that the retention is exclusively governed by partition or adsorption. Therefore, deviations from the expected properties of these functions is an indirect evidence of the existence of other contributions to retention caused for example by steric effects, heterogeneity effects or changes in the conformation of the hydrocarbon chains upon the composition of the mobile phase or even by the collapse of the chains at water rich mobile phases [2,8,9,18,19].

3. Experimental

The liquid chromatography system consisted of a Shimadzu LC-9A pump, a model 7125 syringe loading sample injector fitted with a 20 μl loop (Rheodyne, Cotati, CA, USA), and a UV dector (Shimadzu SPD-10A) working at 254 nm. The reversed-phase chromatographic columns were a C_{18} [250 mm × 4 mm MZ-Analysentechnik column (5 μm Inertsil ODS-3)] and a C_2 [250 mm × 4.6 mm,

Table 1
Retention values ($\ln k$) of non-polar benzene derivatives in methanol–water mobile phases using C_{18} and C_2 column

φ	B	T	EB	PB	*i*PB	*t*BB	t_0 (min)
C_{18} column							
0.00	4.918	6.343	7.693				1.88
0.15	4.197	5.444	6.601				1.85
0.25	3.785	4.912	5.955		6.901		1.84
0.30	3.539	4.592	5.564				1.85
0.35	3.272	4.260	5.173	6.228	5.997	6.681	1.86
0.40	3.008	3.936	4.784	5.766	5.547	6.195	1.88
0.50	2.371	3.155	3.868	4.692	4.500	5.042	1.92
0.60	1.715	2.372	2.956	3.633	3.471	3.915	1.93
0.70	1.054	1.611	2.083	2.625	2.488	2.844	1.95
0.75	0.697	1.199	1.612	2.088	1.964	2.275	1.96
0.80	0.385	0.839	1.200	1.619	1.503	1.778	1.97
0.85	0.020	0.421	0.726	1.078	0.976	1.207	1.98
0.90	−0.324	0.013	0.260	0.552	0.459	0.649	1.99
0.95	−0.686	−0.404	−0.188	0.044	−0.037	0.113	2.00
1.00	−1.067	−0.829	−0.687	−0.514	−0.578	−0.485	2.00
C_2 column							
0.00	3.439	4.856	6.222	7.719	7.496		2.65
0.10	2.970	4.259	5.514	6.995	6.752	7.464	2.60
0.20	2.665	3.784	4.916	6.243	6.018	6.929	2.57
0.30	2.211	3.138	4.074	5.192	4.984		2.56
0.35	1.969	2.806	3.659	4.680	4.420	5.178	2.55
0.40	1.705	2.477	3.245	4.164	3.975	4.613	2.54
0.50	1.128	1.728	2.329	3.047	2.899	3.392	2.53
0.60	0.553	1.029	1.496	2.052	1.935	2.312	2.53
0.70	−0.020	0.328	0.667	1.068	0.976	1.265	2.55
0.75	−0.349	−0.058	0.223	0.556	0.480	0.715	2.57
0.80	−0.619	−0.346	−0.123	0.140	0.078	0.283	2.60
0.85	−0.968	−0.767	−0.581	−0.356	−0.418	−0.217	2.65
0.90	−1.206	−1.040	−0.901	−0.733	−0.790	−0.656	2.66
0.95	−1.537	−1.421	−1.315	−1.214	−1.231	−1.138	2.67
1.00	−1.710	−1.611	−1.560	−1.484	−1.509	−1.471	2.66

B, benzene; T, toluene; EB, ethylbenzene; PB, propylbenzene; *i*PB, isopropylbenzene; *t*BB, *tert*-butylbenzene.

MZ-Analysentechnik column (7 μm Lichrosorb)]. Benzene (B), toluene (T), ethylbenzene (EB), propylbenzene (PB), isopropylbenzene (*i*PB) and *tert*-butylbenzene (*t*BB), were studied from aqueous mobile phases modified with methanol (MeOH), isopropanol (*i*PrOH), acetonitrile (ACN) and tetrahydrofuran (THF). The non-polar benzene derivatives were received from Aldrich and the organic solvents from Merck (grade for liquid chromatography). The obtained experimental data corrected for the extra-column volume and in terms of $\ln k$ versus φ are shown in Tables 1–4. The hold-up time, t_0, was measured by injection of 0.01% KBr [27,28]. These values of t_0 were further verified by indicative measurements using water instead of KBr as the t_0 marker [22]. The values of t_0 determined at each column and eluent composition given in Tables 1–4.

We should point out that there is considerable controversy over the best way of measuring t_0 [27,29] and whether we should use a uniform column hold-up time independent of the modifier concentration or not [30–32]. In addition, the proper determination of the hold-up time of a particular chromatographic system is essential for the correct calculation of solute k values, which is a prerequisite for the evaluation of any theoretical model describing retention in RPLC. For this reason we have analysed our data using both a variable t_0 as given in Tables 1–4 and a uniform one at each column and modifier. For the latter case the data of Tables 1–4 were recalculated using the following t_0 values: Mobile phase (a) methanol–water, $t_0 = 1.95$ min (C_{18}) and 2.53 min (C_2), (b) isopropanol–water, $t_0 = 1.81$ min (C_{18}) and 2.36 min (C_2), (c) acetonitrile–water, $t_0 = 1.61$ min (C_{18}) and 2.30 min (C_2), and (d) tetrahydrofurane–water, $t_0 = 1.40$ min (C_{18}) and 2.21 min (C_2).

4. Data analysis

The whole analysis of data was carried out at Microsoft Excel spreadsheets using the Solver for all linear and non-linear fittings. The first step in the analysis of data was the determination of $\ln f_A^m$ for the whole range of x values used in the present work. Solute activity coefficients, $\ln f_A^m$, were taken from [21]. However, the experimental values of $\ln f_A^m$ have been determined for φ values higher than 0.3 or 0.35, whereas our experimental values of $\ln k$ may reach even the value $\varphi = 0$. Therefore, in order to be able to fit Eq. (1) to our entire data sets there is need of a proper ex-

Table 2
Retention values ($\ln k$) of non-polar benzene derivatives in isopropanol–water mobile phases using C_{18} and C_2 column

φ	B	T	EB	PB	*i*PB	*t*BB	t_0 (min)
C_{18} column							
0.00	4.900						1.88
0.10	4.014	5.229	6.362	7.384	7.121		1.77
0.20	3.625	4.682	5.682	6.567	6.423	6.850	1.74
0.30	2.920	3.693	4.399	5.184	4.996	5.513	1.73
0.40	2.063	2.573	3.034	3.534	3.405	3.732	1.73
0.50	1.346	1.748	2.070	2.410	2.312	2.534	1.74
0.60	0.809	1.127	1.372	1.632	1.552	1.724	1.75
0.70	0.332	0.590	0.773	0.982	0.913	1.040	1.77
0.75	0.109	0.327	0.498	0.667	0.610	0.725	1.79
0.80	−0.101	0.102	0.234	0.391	0.332	0.424	1.80
0.85	−0.278	−0.098	0.010	0.134	0.076	0.161	1.81
0.90	−0.491	−0.341	−0.271	−0.169	−0.222	−0.164	1.82
0.95	−0.635	−0.518	−0.477	−0.417	−0.446	−0.409	1.84
1.00	−0.816	−0.732	−0.719	−0.707	−0.727	−0.707	1.85
C_2 column							
0.00	3.368	4.786	6.153	7.650	7.427		2.65
0.10	2.971	4.107	5.217	6.499	6.237	7.033	2.45
0.15	2.771	3.799	4.815	6.027	5.783		2.40
0.20	2.561	3.480	4.390	5.464	5.243	5.922	2.37
0.30	1.880	2.508	3.114	3.783	3.649	4.065	2.36
0.40	1.052	1.447	1.808	2.192	2.114	2.362	2.36
0.50	0.410	0.665	0.897	1.135	1.094	1.237	2.36
0.60	−0.139	0.039	0.200	0.378	0.352	0.450	2.37
0.70	−0.508	−0.373	−0.269	−0.147	−0.186	−0.106	2.39
0.75	−0.671	−0.567	−0.481	−0.389	−0.411	−0.357	2.40
0.80	−0.790	−0.712	−0.637	−0.584	−0.585	−0.540	2.41
0.85	−0.931	−0.868	−0.827	−0.769	−0.786	−0.746	2.42
0.90	−1.064	−1.014	−0.993	−0.952	−0.961	−0.930	2.44
0.95	−1.126	−1.090	−1.086	−1.054	−1.066	−1.056	2.45
1.00	−1.241	−1.234	−1.235	−1.224	−1.234	−1.228	2.48

Solute symbols defined in Table 1.

trapolation of the values of $\ln f_A^m$ to $\varphi = 0$. For this purpose we applied the following two extrapolation techniques both based on the observation that the plots of $\ln f_A^m$ versus φ exhibit smooth curves with smaller curvature than those of the $\ln f_A^m$ versus x plots.

(a) The first extrapolation technique was based on the following equation:

$$\ln f_A^m = D_0 + D_{1m}\varphi + D_{2m}\varphi^2 \quad (15)$$

using $\varphi \leq 0.8$. Note that D_0 for a certain solute is independent of the modifier, since it is the value of $\ln f_A^m$ in the pure water, whereas D_{1m} and D_{2m} depend upon the modifier used. Thus, according to Eq. (15), for the extrapolation of the experimental $\ln f_A^m$ values of a certain solute in the four modifiers used, we have nine adjustable parameters, D_0, D_{1MeOH}, D_{2MeOH}, ..., D_{2THF}, which can be easily determined by means of the Microsoft Excel Solver. Fig. 1 depicts an example of this extrapolation concerning the $\ln f_A^m$ data of propylbenzene. The values of D_0 obtained are listed in Table 5.

(b) The second extrapolation technique was based on the observation that the plots of $\ln f_A^m$ versus φ in methanol are linear for $\varphi \leq 0.8$. Since the values of D_0 of the various solutes are independent of the modifier, we determined them based on the linear extrapolation:

$$\ln f_A^m = D_0 + D_1\varphi \quad (16)$$

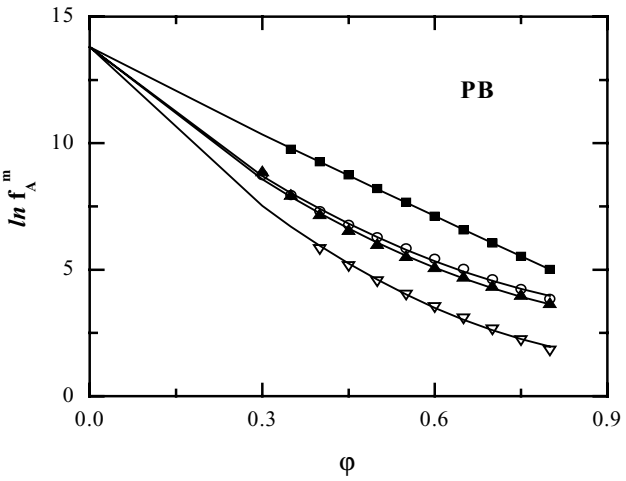

Fig. 1. Extrapolation by means of Eq. (15) of the experimental $\ln f_A^m$ values of PB in MeOH: (■), *i*PrOH (▲), ACN (○) and THF (▽).

Table 3
Retention values ($\ln k$) of non-polar benzene derivatives in acetonitrile–water mobile phases using C_{18} and C_2 column

φ	B	T	EB	PB	*i*PB	*t*BB	t_0 (min)
C_{18} column							
0.00	4.885						1.88
0.10	4.266	5.475	6.622				1.80
0.20	3.696	4.681	5.639	6.719	6.489		1.70
0.30	3.057	3.831	4.579	5.406	5.227	5.746	1.60
0.40	2.407	3.011	3.602	4.269	4.117	4.530	1.55
0.50	1.761	2.249	2.720	3.258	3.130	3.454	1.55
0.60	1.202	1.605	1.991	2.436	2.321	2.596	1.58
0.70	0.692	1.037	1.360	1.740	1.637	1.866	1.60
0.75	0.464	0.787	1.081	1.436	1.339	1.553	1.62
0.80	0.214	0.520	0.792	1.122	1.054	1.224	1.65
0.85	0.024	0.305	0.552	0.856	0.764	0.942	1.66
0.90	−0.251	0.003	0.221	0.493	0.409	0.566	1.70
1.00	−0.973	−0.747	−0.585	−0.366	−0.451	−0.324	1.90
C_2 column							
0.00	3.440	4.856	6.222	7.719	7.496		2.65
0.10	3.174	4.241	5.322	6.546	6.331	7.217	2.44
0.15	2.986	3.910	4.857	5.921	5.736		2.40
0.20	2.789	3.592	4.423	5.345	5.183	5.800	2.35
0.30	2.233	2.833	3.458	4.143	4.021	4.472	2.33
0.40	1.622	2.066	2.526	3.027	2.939	3.270	2.30
0.50	1.085	1.422	1.772	2.154	2.086	2.338	2.30
0.60	0.529	0.763	1.032	1.327	1.292	1.469	2.30
0.70	0.038	0.249	0.457	0.685	0.635	0.800	2.30
0.75	−0.193	−0.016	0.166	0.372	0.334	0.469	2.30
0.80	−0.410	−0.238	−0.097	0.087	0.057	0.179	2.33
0.85	−0.655	−0.528	−0.396	−0.238	−0.268	−0.165	2.36
0.90	−0.916	−0.806	−0.696	−0.561	−0.584	−0.501	2.42
0.95	−1.192	−1.099	−1.004	−0.894	−0.923	−0.844	2.50
1.00	−1.422	−1.305	−1.235	−1.159	−1.193	−1.118	2.60

Solute symbols defined in Table 1.

for $\varphi \leq 0.8$ and using $\ln f_A^m$ data of the various solutes in water–methanol solutions only. The obtained results are also given in Table 5. It is seen that the two methods give almost the same results and this is indirect evidence that the extrapolation gave correct results.

Now $\ln f_A^m$ values for the entire range of x values can be obtained by interpolation using the experimental $\ln f_A^m$ data, the values of $\ln f_A^m(\varphi = 0) = D_0$ obtained from the first extrapolation technique, and a proper equation. Preliminary tests using Eq. (15) showed that this equation is incapable of describing the whole range of $\ln f_A^m$ data, i.e. from $\varphi = 0$–1. For this reason we tested the following two equations:

$$\ln f_A^m = D_0 + D_1 x + D_2 x^2 + D_3 x^3 \quad (17)$$

which has been derived in [1], and

$$\ln f_A^m = a - \frac{bx}{1 + cx} + dx \quad (18)$$

which is an empirical equation. We found that Eq. (17), like Eq. (15), cannot represent satisfactorily the whole range of $\ln f_A^m$ data. In contrast the fitting performance of Eq. (18) is much higher, see for example Fig. 2, and for this reason this equation was used to fit the experimental $\ln f_A^m$ data including the values of $\ln f_A^m(\varphi = 0) = D_0$.

The values of $\ln f_A^m$ for each solute at a certain modifier calculated from Eq. (18) were further used in Eq. (1) to fit the experimental $\ln k$ versus x data.

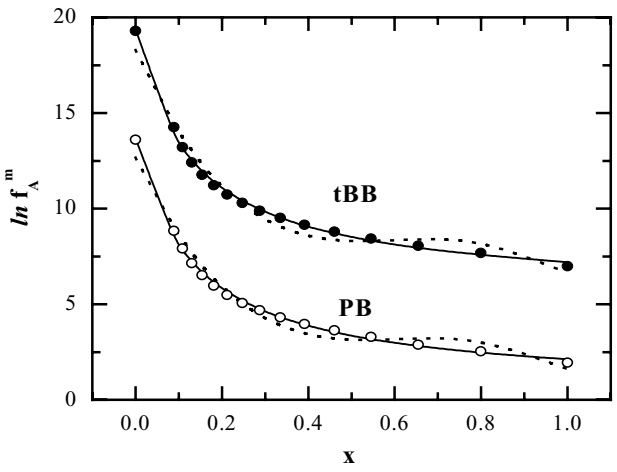

Fig. 2. Plots of $\ln f_A^m$ vs. x for PB (○) and *t*BB (●) in *i*PrOH. Points are experimental data, lines were calculated from the best fits of Eq. (17) (- - -) and Eq. (18) (—). Data of *t*BB are shifted along the y-axis by 5.

Table 4
Retention values ($\ln k$) of non-polar benzene derivatives in tetrahydrofurane–water mobile phases using C_{18} and C_2 column

φ	T	EB	PB	*i*PB	*t*BB	t_0 (min)
C_{18} column						
0.00						1.88
0.10	5.334	6.419		7.398		1.66
0.15	5.061	6.061	7.208	6.959		1.62
0.30	3.765	4.385	5.056	4.920	5.395	1.44
0.40	2.705	3.119	3.560	3.468	3.772	1.41
0.50	1.861	2.143	2.444	2.383	2.577	1.40
0.60	1.180	1.377	1.588	1.542	1.669	1.39
0.70	0.585	0.712	0.853	0.779	0.880	1.37
0.75	0.327	0.433	0.546	0.478	0.578	1.39
0.80	0.037	0.120	0.205	0.185	0.255	1.42
0.85	−0.216	−0.174	−0.134	−0.140	0.126	1.44
0.90	−0.511	−0.489	−0.496	−0.468	−0.333	1.47
0.95	−0.895	−0.903	−0.892	−0.890	−0.617	1.53
1.00	−1.371	−1.411	−1.425	−1.437	−0.771	1.67
C_2 column						
0.00	4.786	6.153	7.650	7.427		2.65
0.10	4.507	5.559	6.770	6.698	7.342	2.42
0.20	3.921	4.744	5.678	5.506	6.124	2.28
0.30	3.018	3.588	4.212	4.105	4.525	2.22
0.40	2.054	2.436	2.831	2.772	3.036	2.20
0.50	1.264	1.526	1.808	1.756	1.937	2.20
0.60	0.590	0.769	0.954	0.930	1.054	2.21
0.70	0.010	0.132	0.248	0.246	0.321	2.21
0.75	−0.225	−0.132	−0.039	−0.043	0.022	2.22
0.80	−0.529	−0.455	−0.399	−0.392	−0.345	2.25
0.85	−0.815	−0.765	−0.728	−0.723	−0.689	2.30
0.90	−1.127	−1.091	−1.068	−1.063	−1.044	2.36
0.95	−1.543	−1.543	−1.543	−1.534	−1.534	2.48
1.00	−2.337	−2.381	−2.427	−2.406	−2.406	2.65

Solute symbols defined in Table 1.

Table 5
Values of $\ln f_A^m(\varphi = 0) = D_0$ obtained from two extrapolation techniques using Eq. (15) or (16)

	Solute*					
	B	T	EB	PB	*i*PB	*t*BB
Eq. (15)	9.04	10.5	12.1	13.6	13.3	14.3
Eq. (16)	9.01	10.4	11.8	13.5	13.0	14.2

* Solute symbols defined in Table 1.

5. Results and discussion

The results from the curve fitting procedures and in particular the standard deviations σ of the fits are given in Table 6. It is seen that only the data from the C_{18} column are fitted satisfactorily to the theoretical Eq. (1). In contrast, the standard deviations of the fits concerning the C_2 column are systematically higher than those of the C_{18} column, in complete agreement with our assumption that the partition mechanism cannot be valid for C_2 columns due to the lack of available cavities in the stationary phase of a C_2 column. The comparison between experimental and calculated $\ln k$ values in $\ln k$ versus x plots verifies the above result. For example, in Fig. 3 we observe that the deviations between experimental and calculated $\ln k$ values when we use a C_2 column may be rather small but they are systematic, leaving no doubt that the partition model is inadequate for this column.

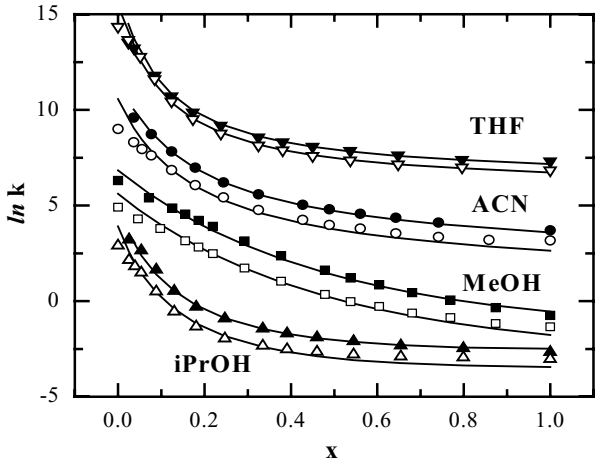

Fig. 3. Plots of $\ln k$ vs. x for T in four mobile phases using a C_{18} (closed symbols) and a C_2 (open symbols) column. Points are experimental data calculated using a uniform t_0 value per column and modifier, lines were calculated from the best fits of Eq. (1). Data in *i*PrOH, ACN and THF are shifted along the y-axis by −2, +4 and +8, respectively.

Table 6
Values of the standard deviation σ of the fit to Eq. (1)

Column	Solute											
	B[a]	B[b]	T[a]	T[b]	EB[a]	EB[b]	PB[a]	PB[b]	*i*PB[a]	*i*PB[b]	*t*BB[a]	*t*BB[b]
MeOH–water												
C_{18}	0.189	0.193	0.197	0.208	0.219	0.229	0.093	0.123	0.168	0.204	0.229	0.262
C_2	0.377	0.328	0.294	0.252	0.261	0.225	0.266	0.222	0.193	0.171	0.146	0.152
C_2[c]	0.377	0.328	0.294	0.252	0.261	0.225	0.216	0.141	0.145	0.101	0.101	0.107
*i*PrOH–water												
C_{18}	0.178	0.197	0.150	0.168	0.192	0.210	0.235	0.250	0.242	0.259	0.242	0.259
C_2	0.405	0.391	0.370	0.356	0.370	0.357	0.383	0.372	0.346	0.335	0.330	0.297
C_2[c]	0.405	0.391	0.255	0.211	0.249	0.206	0.288	0.254	0.263	0.229	0.345	0.310
ACN–water												
C_{18}	0.328	0.395	0.143	0.188	0.125	0.163	0.085	0.100	0.072	0.103	0.086	0.099
C_2	0.543	0.571	0.497	0.528	0.458	0.484	0.370	0.406	0.365	0.398	0.192	0.104
C_2[c]	0.543	0.571	0.301	0.306	0.257	0.265	0.095	0.072	0.085	0.065	0.182	0.058
THF–water												
C_{18}			0.066	0.140	0.262	0.304	0.130	0.139	0.223	0.246	0.080	0.099
C_2			0.438	0.814	1.218	0.818	0.788	0.787	0.754	0.758	0.266	0.372
C_2[a]			0.148	0.419	0.698	0.389	0.183	0.209	0.299	0.303	0.093	0.322

[a] σ has been calculated using a uniform t_0 value per column and mobile phase.
[b] σ has been calculated using variable t_0.
[c] σ has been calculated at the same x values with those used for the C_{18} column.

However, the above comparison about the fitting performance of Eq. (1) for the two columns is useful only if we use data sets with the same x values. From Tables 1–4 we observe that the data sets for the C_2 column are more complete towards the low x (or φ) values. Thus we proceeded to refit Eq. (1) to those data of the C_2 column that have the same x values with those of the C_{18} column. Note that in some cases we had to use interpolation to succeed data sets of the same x values. The "new" data sets of the C_2 column gave better fits, which show that the partition model seems to describe the retention of several solutes in the C_2 column. This result is obviously incorrect from a physical

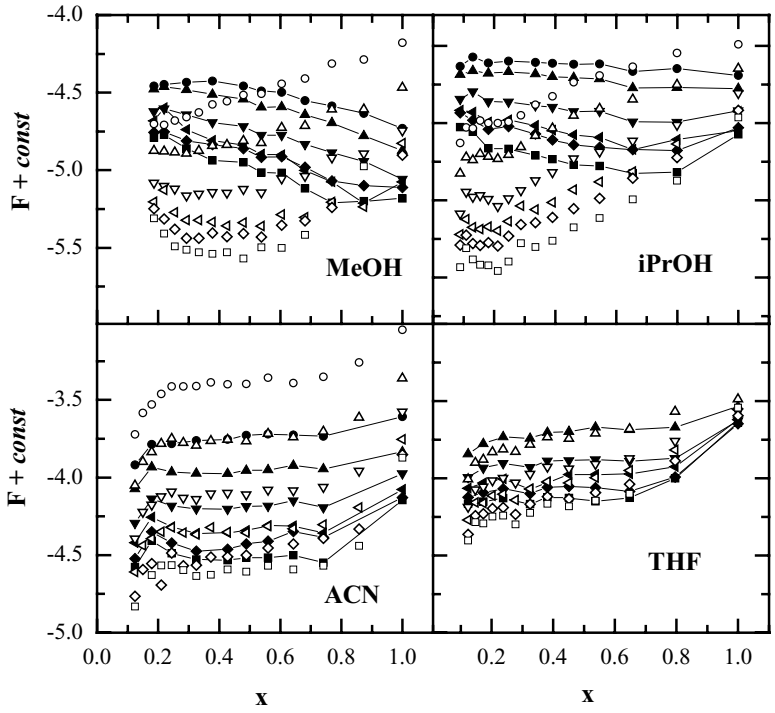

Fig. 4. Plots of F vs. x for B (●, ○), T (▲, △), EB (▼, ▽), *i*PB (◀, ◁), PB (◆, ◇) and *t*BB (■, □) at each of the four modifiers using a C_{18} (closed symbols connected with lines) and a C_2 (open symbols) column. Data correspond to a uniform hold-up time per column and modifier.

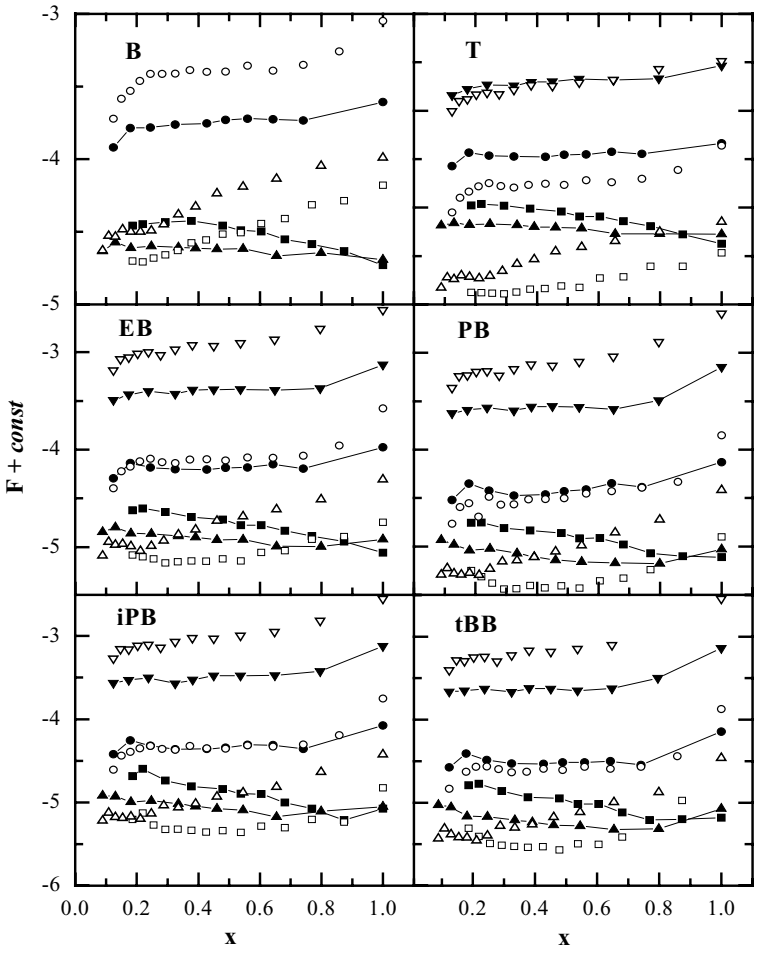

Fig. 5. Plots of F vs. x for each solute shown in the figure in the four modifiers, ACN (●, ○), THF (▽, ▼), MeOH (■, □) and *i*PrOH (△, ▲) using a C_{18} (closed symbols connected with lines) and a C_2 (open symbols) column. Data correspond to a uniform hold-up time per column and modifier.

point of view and shows clearly that the fitting procedure is not sensitive enough to clarify the origin of the retention mechanism.

For this reason we proceeded to clarify the retention mechanism in C_{18} columns through the F versus x, S versus x and $P(\lambda)$ versus x plots based on their properties described in the theoretical section. These plots are shown in Figs. 4–8.

Plots of F versus x are shown in Figs. 4–6. Fig. 4 shows the F versus x plots of all solutes at each of the four modifiers used in the present study. These plots have been calculated using a uniform hold-up time per column and modifier. The use of a variable hold-up time leads to similar plots, except for the case of water–THF solutions, where a notable increase in the curvature of the F–x plots is observed. It is seen that there is a clear differentiation of these plots on passing from C_2 to C_{18} column in mobile phases modified by *i*PrOH and MeOH. Especially the F versus x plots in water–*i*PrOH solutions become almost linear and parallel to the x-axis only in the C_{18} column. This is strong evidence that the partition mechanism should predominate in this eluent. In the presence of MeOH in the mobile phase, the plots of F versus x are quite different in the two columns; they become, within the experimental error, linear in the C_{18} column but the lines are not parallel to the x-axis. This may show the validity of the partition mechanism affected by some strong contribution coming from steric effects or changes in the conformation of the hydrocarbon chains upon the composition of the mobile phase [2,8,9]. In what concerns the mobile phases modified by ACN and THF, we observe that there is not a significant differentiation in the F versus x plots on passing from the C_2–C_{18} column. The F versus x plots are almost linear and parallel to the x-axis for both columns at least in the region from $x = 0.2$ to 0.8. However, since the partition mechanism is ruled out from C_2 columns, the parallelism of these plots to the x-axis cannot be an indication of the validity of this mechanism for the C_{18} column. The similarity of the F versus x plots for the two columns might show that the behaviour of the C_2 and C_{18} columns is almost similar in the presence of ACN or THF, which, if true, means that in these cases the adsorption mechanism should predominate to the retention mechanism.

The above evidences about the retention mechanism are further strengthening from the F versus x plots of each

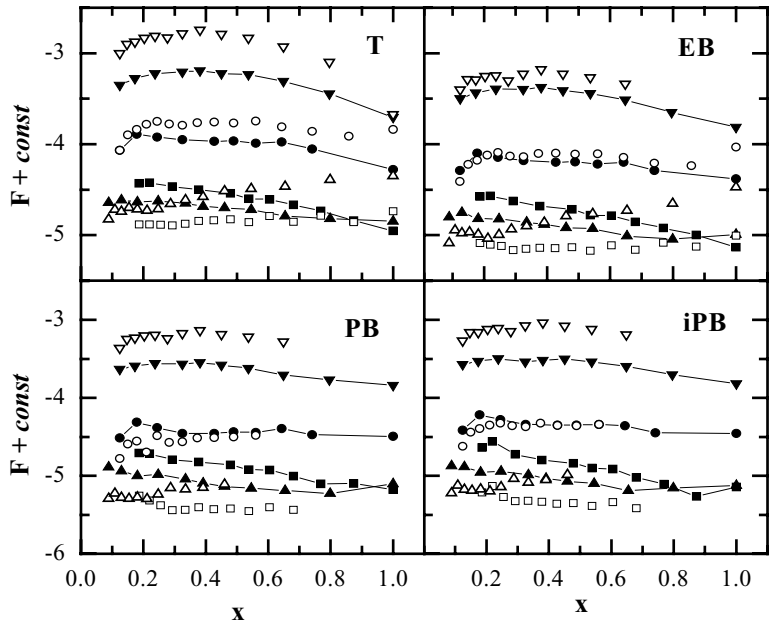

Fig. 6. As in Fig. 5 but for variable hold-up time.

solute in the four mobile phases depicted in Figs. 5 and 6. The plots of these figures rule out any possibility that the partition mechanism holds in all four modifiers used in the present work. If this were the case, then the plots of F versus x in the C_{18} column for each solute in various modifiers would coincide to one straight line parallel to the x-axis. It is seen that this does not happen and only the F versus x in the presence of *i*PrOH and MeOH tend to coincide at x values higher than 0.7. Therefore, if we take into account the conclusion drawn above from Fig. 4 that the partition mechanism should predominate in C_{18} columns when the mobile phase is modified with *i*PrOH, then the above fact

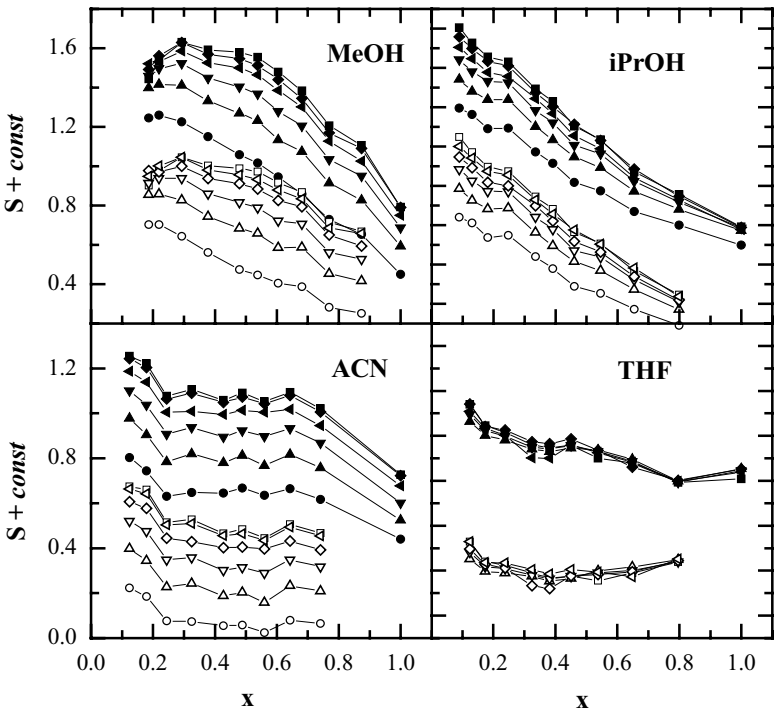

Fig. 7. Plots of S vs. x for B (●, ○), T (▲, △), EB (▼, ▽), *i*PB (◀, ◁), PB (◆, ◇) and *t*BB (■, □) in the four modifiers shown in the figure. Data correspond to a uniform (closed symbols) and a variable (open symbols) hold-up time.

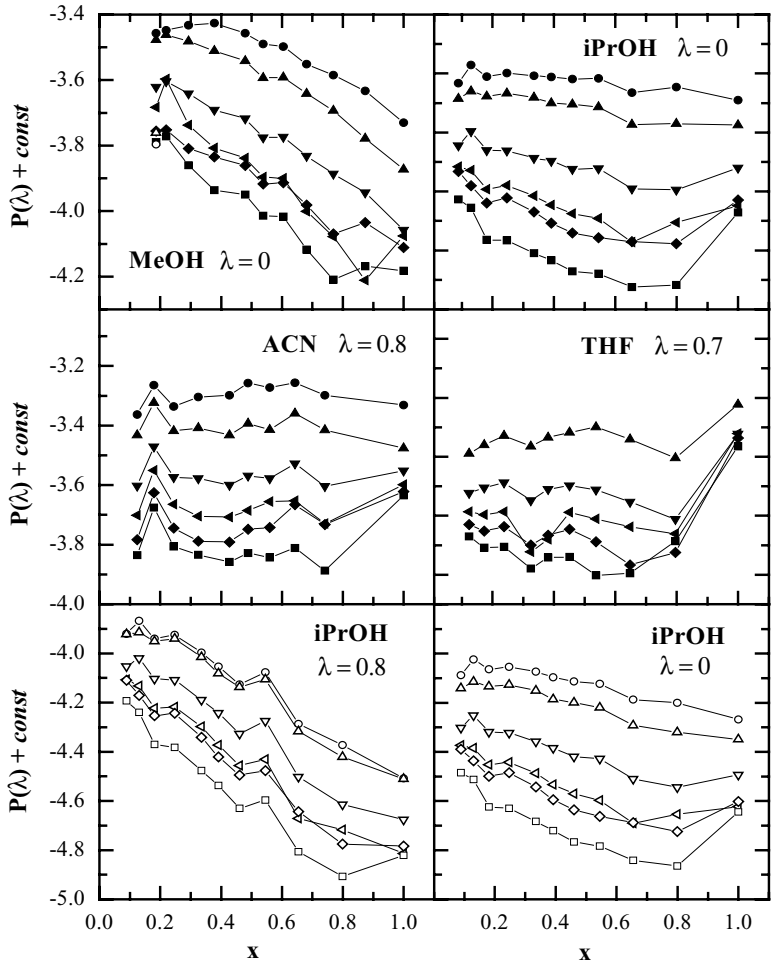

Fig. 8. Plots of $P(\lambda)$ vs. x for B (●, ○), T (▲, △), EB (▼, ▽), *i*PB (◀, ◁), PB (◆, ◇) and *t*BB (■, □) in the four modifiers and at λ values shown in the figure. Data correspond to a uniform (closed symbols) and a variable (open symbols) hold-up time.

might show that this mechanism is likely to be valid at least in MeOH rich solutions.

A similar picture about the retention mechanism in C_{18} columns is obtained from the S versus x plots shown in Fig. 7. It is seen that these plots are perfectly linear and parallel to the x-axis in mobile phases modified by ACN in the region $0.2 \leq x \leq 0.7$ and roughly linear and parallel to the x-axis in the presence of THF. In contrast, they exhibit a strong dependence on x in the presence of MeOH and especially of *i*PrOH. Therefore, the adsorption mechanism is likely to have a significant contribution to the retention in the presence of ACN and THF, whereas this mechanism cannot be valid in mobile phases of *i*PrOH–water solutions. The behaviour of MeOH as modifier arising from the plots of S versus x is rather complicated. It might be close to that of *i*PrOH, although for certain solutes and in particular for propyl-, isopropyl- and *tert*-butylbenzene the adsorption might determine the retention at $x < 0.6$.

Finally, the plots of $P(\lambda)$ versus x become roughly linear and parallel to the x-axis when $\lambda = 0$ in the presence of *i*PrOH and $\lambda = 0.8$ and 0.7 in the presence of ACN and THF, respectively (Fig. 8). Therefore, these plots confirm the above results about the retention mechanism in octadecyl reversed-phase columns in the presence of *i*PrOH, ACN and THF. In what concerns MeOH, we could not find a value $\lambda \geq 0$ to make these x plots parallel to the x-axis. According to the arguments presented in the theoretical part, deviations from the expected behaviour is an indirect evidence of the existence of contributions to retention coming from steric effects, heterogeneity effects or changes in the conformation of the stationary phase. Unfortunately these deviations do not help to an unambiguous clarification of the retention mechanism.

In order to examine further the validity of the above results and conclusions we applied the suggested criteria to two data sets from literature. In particular, we examined the retention behaviour of benzene, toluene, ethylbenzene and propylbenzene in a LiChrospher 100 RP-18 column using the data reported by Bosch et al. [33] and in a Hypersil ODS column using Cheong and Carr's data [22]. If the case of MeOH is excluded, the results of the application of the tests suggested in this paper to the literature data are in general similar to the above results indicating both the validity of these results and their independence of the particular type of

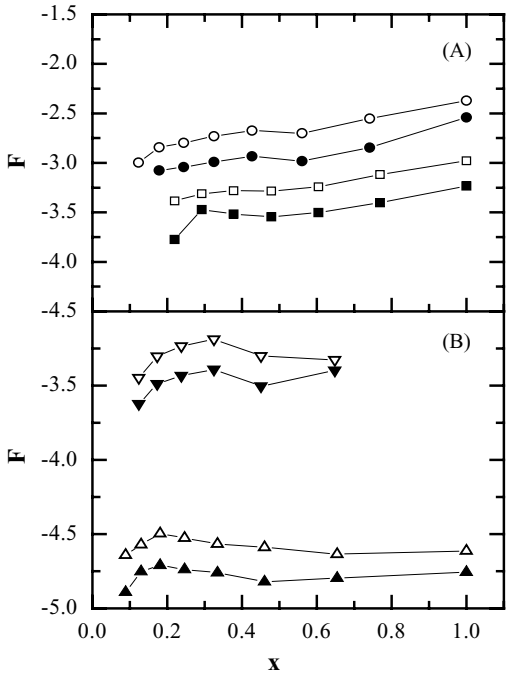

Fig. 9. Plots of F vs. x for T (open symbols) and EB (closed symbols) in the four modifiers, ACN (●, ○), THF (▽, ▼), MeOH (□, ■) and *i*PrOH (△, ▲) using (A) a LiChrospher 100 RP-18 and (B) a Hypersil ODS column. Data were taken from [22,33].

the reversed-phase column. Some of the tests are shown in Figs. 9 and 10. Fig. 9 shows plots of F versus x for toluene and ethylbenzene in the four modifiers. Note the similarity of these plots with the corresponding ones in Fig. 5 and the fact that the distance of the F versus x curves for both toluene and ethylbenzene in MeOH and ACN is about 0.6, a value that is equal to the corresponding distance found in our plots of Figs. 5 and 6. Also this distance in THF and *i*PrOH is about 1.4 in both Figs. 5 and 9. This is in line with our results, which exclude the possibility that the partition mechanism holds in all four modifiers used in the present work. In addition, Fig. 10 shows striking similarities with the corresponding Fig. 7, except for the case where MeOH is used as modifier.

Therefore, the retention of small and non-polar solutes in C_{18} columns should be due to partition in the presence of *i*PrOH in the mobile phase, whereas significant contributions from the adsorption mechanism are likely in mobile phases modified by ACN and THF. That is, the retention of the compounds studied in C_{18} columns and in the presence of ACN or THF as modifiers is likely due to a combined adsorption-partition mechanism with the dominant contribution coming from adsorption. The behaviour of the C_{18} columns in the presence of methanol is rather obscure. The plots of S versus x using data from literature show that MeOH favours the adsorption mechanism (Fig. 10), whereas our data in Fig. 7 do not exclude the partition mechanism at least in methanol rich mobile phases. In general our results show that the retention mechanism in the presence of MeOH is affected possibly by a continuous change in the conformation of the hydrocarbon chains of the C_{18} columns from partially to fully extended configurations following the increase in methanol concentration up to $x = 0.7$ [2,8,9]. This explains the fact that the plot F versus x of a certain solute

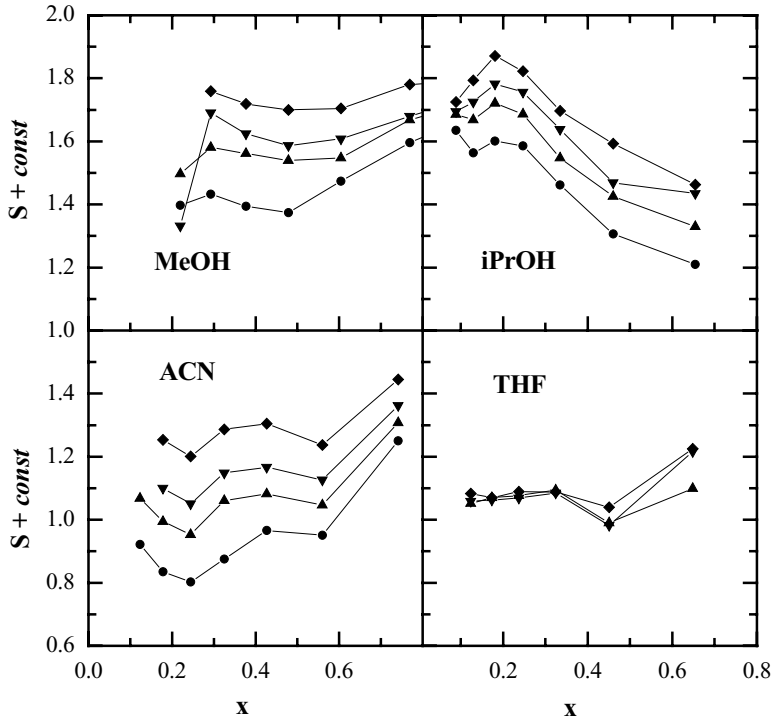

Fig. 10. Plots of S vs. x for B (●), T (▲), EB (▼) and PB (◆) in the four modifiers shown in the figure. They have been calculated from data obtained by means of a LiChrospher 100 RP-18 column (MeOH, ACN) [33] and a Hypersil ODS column (*i*PrOH, THF) [22].

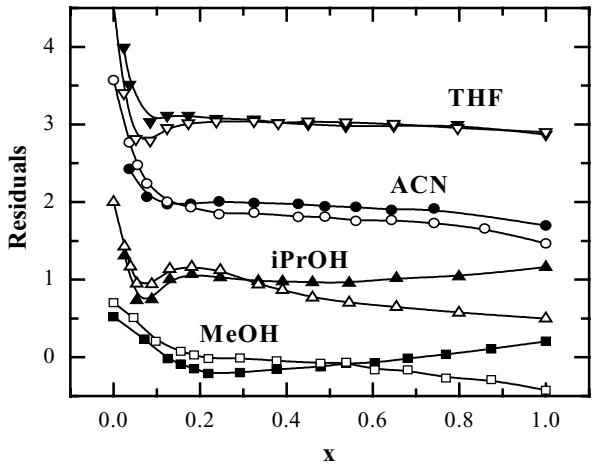

Fig. 11. Residual plots of the fittings of Fig. 3. Data of *i*PrOH, ACN and THF are shifted along the *y*-axis by 1, 2 and 3, respectively.

in MeOH tends to coincide with the corresponding plot in the presence of *i*PrOH at $x \geq 0.7$ (Figs. 5 and 6). At any rate the conflicting the results of the various tests show that more studies using various types of C_{18} and C_2 columns are necessary for the complete clarification of the behaviour of MeOH as modifier.

We should point out that the above results about the retention mechanism hold for aqueous mobile phases modified with *i*PrOH, MeOH, ACN and THF at concentrations higher than about $x = 0.1$–0.2. At concentrations of the organic modifier lower than this limit, i.e. at water rich solutions, the bonded phase is collapsed or at least the chains are not fully extended towards the mobile phase [2,8,9,18,19]. Therefore, in this region of x values the partition mechanism is very unlikely in all modifiers. Indirect evidence that this view is correct comes from the residual plots of the fitting procedure. Fig. 11 shows the residual plots of the fittings of Fig. 3. It is seen that the shape of these plots at $x \leq 0.2$ is the same for the two columns. It is also the same for the two columns in the presence of ACN and THF throughout the region of x values. That is, the shape of the residual plots is the same for the two columns in all cases where the adsorption mechanism prevails or it has a significant contribution to the retention. The shape of these plots becomes different for the two columns in the presence of MeOH (at a rather high x value, $x > 0.55$) as well as in the presence of *i*PrOH ($x > 0.3$), i.e. in the case where our tests show the domination of the partition mechanism.

6. Conclusions

For the elucidation of the retention mechanism in reversed-phase chromatographic columns we have suggested a new methodology based on a systematic comparison of the retention properties of a solute in C_{18} and C_2 columns under the assumption that the retention in C_2 columns is governed by adsorption phenomena on the hydrocarbon chains. The comparison involves usual curve fitting procedures but also three new tests based on the properties of the F, S and $P(\lambda)$ functions defined in this paper. Our results are encouraging and it is likely that the method proposed here would become a tool for a better understanding of retention processes in RPLC. In particular, we found that the elucidation of the retention mechanism should not be based only on curve fitting procedures, like those adopted in [1] as well as in the present paper. They are not sensitive enough and for this reason they may lead to unreliable results. For example, this approach shows that for solutes with small and non-polar molecules the dominant retention mechanism in reversed-phase C_{18} chromatographic columns is the partition. However, the use of the combination of the more accurate tests based on the properties of the F, S and $P(\lambda)$ functions show that that retention mechanism for these solutes depends on the organic modifier used. There is a rather significant contribution from adsorption in mobile phases modified by ACN and THF, whereas the partition is likely to predominate in *i*PrOH–water solutions at $x > 0.2$. The case of MeOH needs further studies, because the various tests applied to our data and data taken from literature do not clarify unambiguously the behaviour of MeOH as modifier.

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