Journal of Chromatography, 595 (1992) 53–61
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 891

Validation of chromatographic retention models in reversed-phase high-performance liquid chromatography by fitting experimental data to the relevant equations

Nina Sadlej-Sosnowska

Drug Institute, 30/34 Chełmska Str., 00-725 Warsaw (Poland)

Irma Śledzińska

Institute of Physics, Warsaw University of Technology, 75 Koszykowa Str., 00-662 Warsaw (Poland)

(First received January 3rd, 1991; revised manuscript received October 8th, 1991)

ABSTRACT

Capacity factors (k') were measured for five steroid hormones in the binary mobile phases methanol–water and acetonitrile–water as a function of methanol and acetonitrile concentration. The results were interpreted in terms of five models of chromatographic retention selected from the many that have been published. The R factor statistic based on the F distribution was used for testing the goodness of fit of the models to the experimental data.

INTRODUCTION

The development of chromatographic methodology and the accumulation of numerous experimental data have been continuously accompanied by interest in the theory of chromatographic retention. This has resulted in various simplified models of the mutual interactions between stationary phase, solutes to be chromatographed and components of the mobile phase. Such chromatographic systems are relatively complicated, so any model can reflect only some aspects of the physico-chemical processes significant to the retention behaviour of a given solute in its environment. These models are most frequently characterized by the functional dependence between capacity factor, k', and the composition of the mobile phase. In reversed-phase high-performance liquid chromatography (RP-HPLC), the mobile phase is a mixture of water and a miscible organic solvent ("modifier"); the composition of the mobile phase is expressed by the volume fraction φ or mole fraction x of the latter. Various models anticipate different relationships connecting k' with φ or x, and the relevant constants have different physical meaning.

Authors of individual approaches or researchers interpreting their experimental results in terms of a given model may state that the goodness of fit of the experimental data to a functional dependence $k'(\varphi)$ or $k'(x)$ supports the theoretical premises on which this dependence is based. Such an implication seems to be well founded. Nevertheless, attempts to interpret any given set of data using more than one theoretical approach and to compare the results are lacking. Among many models and consequently mathematical relationships reported in the literature, we have selected five: (A) Snyder–Soczewiński displacement model [1–3], assuming monolayer adsorption of the solute or solvent on the stationary phase; (B) Scott–Kucera model [4,5] assuming also monolayer adsorption and taking into account dispersion interactions between the solvent and

0021-9673/92/$05.00

solute in the mobile phase; (C) model of Jaroniec and co-workers [6,7], taking into consideration self-association of one component of a binary mobile phase and association of solute molecules with that component; (D) the most universally adopted in RP-LC exponential relationship for $k'(\varphi)$ introduced first for partition chromatography [8,9], assumed to be empirical [10] but it can be justified by consideration of the partition ratio of a substance between the stationary and mobile phases using the Kemula–Buchowski equation [8]; and (E) the Dill lattice statistical thermodynamic theory [11,12] underlying the affinity of the solute for the grafted chains. The same functional (quadratic) dependence was deduced by Schoenmakers and co-workers [13–15]; they introduced solubility parameters for the calculation of intermolecular forces, based on the Hildebrand theory of regular solutions. The same expression was derived by Jandera *et al.* [16]; their model also emphasizes intermolecular interactions between the solute and the mobile phase.

Models A, B and C were elaborated for normal-phase chromatographic systems but we have tried to check them also in RP-LC.

The above-mentioned approaches yield the following functional dependences between k' and φ or x:

$$\text{(A)}\quad \log k' = -(A_s/n_B)\log x + \log k'_B \qquad (1)$$

$$\text{(B)}\quad 1/k' = A' + B'\varphi \qquad (2)$$

$$\text{(C)}\quad 1/k'x = \alpha + \beta x \qquad (3)$$

$$\text{(D)}\quad \log k' = -S\varphi + \log k'_w \qquad (4)$$

$$\text{(E)}\quad \log k' = a\varphi^2 + b\varphi + c \qquad (5)$$

Some of the parameters have well defined physical meanings, *e.g.*, k'_B in eqn. 1 is the capacity factor in pure organic modifier, k'_w the capacity factor in pure water, A_s the surface area of the adsorbed solute molecule and n_B the surface area of the adsorbed molecule of organic modifier; more complex interpretations of other parameters have been given.

The usefulness of eqns. 1–4 for the interpretation of experimental data was checked by plotting them with the use of coordinates that ought to produce a linear plot. For example, representation of experimental points by functional eqn. 1 ought to give a straight line if the coordinates ($\log k'$, $\log x$) are used.

Eqn. 1, elaborated primarily for adsorption chromatography and applied in such systems [17], has also been used for RP bonded phases in the analysis of proteins [18]. Eqns. 2 and 3 (similarly to eqn. 1) were devised for adsorption, normal-phase chromatography, and to our knowledge have not been applied to RP systems. Eqn. 4 has been successfully applied to the interpretation of chromatographic data in numerous papers [10,19–29]; in most of them the relationship $\log k' = f(\varphi)$ was linear, with few exceptions. The quadratic function in eqn. 5 described the retention of various aromatics [13], including amines and phenols.

Apart from the relationships mentioned above, one can find in the literature many more theoretical approaches and methods of chromatographic data interpretation, *e.g.*, those relating retention to the polarity of the mobile phase [30] and more rigorous models [31] introducing a number of physico-chemical constants that are difficult to determine.

Taking into consideration that the same set of data could be satisfactorily described by different authors by two entirely different approaches [28,32], we decided to try to establish how our own results would fit the several models quoted above. For this purpose we chose a group of five steroid hormones: prednisolone (**1**), hydrocortisone (**2**), methylprednisolone (**3**), testosterone (**4**) and progesterone (**5**).

EXPERIMENTAL

HPLC system

The HPLC system used was a Pye Unicam PU 4100 (pump, oven and UV detector), with a Varian fluorescence detector (for the measurement of void volume, V_0) and Shimadzu R6A integrator. The chromatograms of steroid compounds were monitored at 240 nm. The column used was Partisil 10 ODS (25 cm × 4.6 mm I.D.), maintained at 35 or 22°C with a flow-rate of 1.5 ml/min. V_0 was determined by two techniques: (1) as proposed by Neidhart *et al.* [33], involving the doping of the mobile phase with a fluorophore (quinine sulphate), injection of undoped mobile phase and measuring the decrease in fluorescence; by this method V_0 was determined separately in each mobile phase; and (2) a static method, giving V_0 (static), in which the column was successively filled with two solvents of different density (carbon tetrachloride and isopropanol) and weighed after each filling [34]. This method gives the maximum value of V_0 (total column porosity). The dead volume was measured after switching the column out of the system and connecting the detector directly with the injection valve. It was equal 0.13 ml both for UV and fluorescence detectors.

Sample preparation

The steroid concentrations were 4 μg/ml ± 10%, always prepared in a given mobile phase (methanol–water or acetonitrile–water) of varying composition.

RESULTS AND DISCUSSION

Determination of the capacity factors, k′

For the five steroid hormones, k' values were investigated with several methanol–water and acetonitrile–water mobile phase compositions. For the calculation of k' (and other dependent parameters) it is necessary to determine V_0, yet there is no universally accepted method for the accurate measurement of this value. The variety of methods for V_0 determination were reviewed by Smith *et al.* [35]; most of them were tried by us. The method proposed by Neidhart *et al.* [33] seemed to be the most reliable. V_0 decreased with increasing fraction of organic modifier, according to the predictions of the statistical mechanical theory of retention [36]. For acetonitrile–water it ranged from 2.86 to 2.64 ml and for methanol–water from 2.85 to 2.80 ml. The static void volume, V_0 (static), the upper limit of V_0, was also determined and used for further calculation because it is well defined and does not depend on the composition of the mobile phase; the results of all other methods for V_0 determination depend not only on the mobile phase composition but also on the substance chosen as a void volume marker. V_0 (static) was equal 3.03 ml. Of the other methods used for V_0 determination, the breakthrough and minor disturbance methods with methanol–water mixtures gave the results which, within experimental error, were identical with the value obtained by static method, but with acetonitrile–water mixtures the breakthrough method, at low acetonitrile concentrations, gave results higher than the upper (static) limit, indicating retention of acetonitrile in the system; at higher acetonitrile concentrations the values obtained by the breakthrough method were,

TABLE I

k' VALUES FOR STEROID HORMONES ON PARTISIL ODS WITH METHANOL–WATER PHASES AT 35°C

φ = Methanol volume fraction; x = methanol mole fraction.

Compound	V_0										
	$\varphi = 0.30$ $x = 0.160$	0.35 0.193	0.40 0.229	0.45 0.267	0.50 0.308	0.55 0.352	0.60 0.400	0.65 0.453	0.70 0.509	0.75 0.572	0.80 0.640
1	14.5	7.83	4.46	2.62	1.32	1.00	0.72				
2	15.3	8.26	4.71	2.76	1.41	1.06	0.79				
3		14.5	7.79	4.34	2.03	1.50	0.98	0.64			
4				15.1	7.95	4.62	2.93	1.81	1.26	0.89	
5					17.2	8.94	5.19	2.97	1.90	1.25	0.85
	V_0 (static)										
	$\varphi = 0.30$ $x = 0.160$	0.35 0.193	0.40 0.229	0.45 0.267	0.50 0.308	0.55 0.352	0.60 0.400	0.65 0.453	0.70 0.509	0.75 0.572	0.80 0.640
1	13.6	7.31	4.14	2.40	1.17	0.87	0.60				
2	14.4	7.71	4.37	2.54	1.25	0.93	0.66				
3		13.6	7.27	4.03	1.84	1.34	0.85	0.52			
4				14.2	7.38	4.26	2.66	1.61	1.09	0.75	
5					16.0	8.30	4.76	2.69	1.68	1.08	0.71

within experimental error, identical with those of the method proposed by Neidhart *et al.* [33].

Taking into consideration the above results, we decided to calculate values of k' with double V_0 data, as mentioned under Experimental, and to see if the results of fitting k' to eqns. 1–5 would depend on the choice of V_0. The experimental data are given in Tables I and II.

Statistical analysis of data

The experimental k' values were fitted to eqns. 1–5 using the least-squares method (linear regression). The resulting parameters and correlation coefficients r are given in Tables III–VII. Standard deviations of the parameters are given in parantheses and correspond to the last digit.

A high value of the correlation coefficient r is often considered as validation of an assumed equation. For example, Jaffé [37] arbitrarily classified the goodness of fit into the categories excellent, good (satisfactory) and fair, corresponding to r values of at least 0.99, 0.95 and 0.90, respectively. The tabulated r values are all >0.95 and in most instances >0.99 (Tables III–VII).

However, it was pointed out by Ehrenson *et al.* [38] that the correlation coefficient gives a non-linear acceptability scale, with good and bad correlations often crowded in the range 0.9–1.0, particularly for small samples. Therefore, the conclusions based on this value should be treated with caution.

The R factor statistic, extensively used among crystallographers [39] and employed by Ehrenson [40] for free energy relationship fitting, seems to be a more appropriate measure of the goodness of fit. The generalized R factor [41] is defined as

$$R = \left[\frac{\sum\limits_i (f_i^{\text{obs}} - f_i^{\text{calc}})^2 \, \omega_{ii}}{\sum\limits_i (f_i^{\text{obs}})^2 \, \omega_{ii}} \right]^{1/2} \qquad (6)$$

where f^{obs} are a set of observed functions and f^{calc} are a set of calculated values for them in some parametrized model representation; ω_{ii} are elements of a diagonal weight matrix and are proportional to the inverse squares of the corresponding uncertainties of the experimental f_i^{obs} values. The statistical character of the chromatographic processes and

TABLE II

k' VALUES FOR STEROID HORMONES ON PARTISIL ODS WITH ACETONITRILE–WATER MOBILE PHASES AT 22°C

φ = Acetonitrile volume fraction; x = acetonitrile mole fraction.

Compound	V_0												
	$\varphi = 0.18$ $x = 0.070$	0.20 0.079	0.24 0.098	0.28 0.113	0.32 0.139	0.36 0.162	0.40 0.186	0.44 0.212	0.48 0.241	0.52 0.271	0.56 0.304	0.60 0.340	0.64 0.379
1	13.9	9.57	4.94	3.06	2.03	1.42	1.02						
2	14.2	9.99	5.18	3.20	2.12	1.49	1.09						
3		18.1	8.61	5.06	3.13	2.10	1.46	1.07					
4					11.4	7.24	4.77	3.31	2.55	1.95	1.58		
5							12.6	7.91	5.61	3.98	2.98	2.38	1.84
	V_0 (static)												
	$\varphi = 0.18$ $x = 0.070$	0.20 0.079	0.24 0.098	0.28 0.113	0.32 0.139	0.36 0.162	0.40 0.186	0.44 0.212	0.48 0.241	0.52 0.271	0.56 0.304	0.60 0.340	0.64 0.379
1	13.2	8.84	4.38	2.63	1.68	1.14	0.78						
2	13.5	9.23	4.60	2.76	1.76	1.20	0.84						
3		16.8	7.71	4.43	2.66	1.75	1.17	0.82					
4					10.0	6.30	4.09	2.80	2.11	1.59	1.24		
5							11.0	6.85	4.79	3.36	2.46	1.94	1.47

TABLE III

CONSTANTS OF THE FUNCTIONAL RELATIONSHIP BETWEEN k' AND x, CALCULATED BY SUBSTITUTING DATA FROM TABLES I AND II INTO EQN. 1

r = Linear regression correlation coefficient; meaning of $\mathscr{R}$ is explained in the text.

Compound	V_0							
	Methanol				Acetonitrile			
	Log k'_B	A_s/n_B	r	$\mathscr{R}$	Log k'_B	A_s/n_B	r	$\mathscr{R}$
1	−1.53(7)	3.4(1)	0.997	1.02	−1.96(7)	2.66(7)	0.998	4.16
2	−1.49(7)	3.4(1)	0.997	1.11	−1.92(7)	2.65(7)	0.998	5.26
3	−1.49(7)	3.7(1)	0.997	1.24	−1.91(7)	2.84(8)	0.998	4.05
4	−0.99(5)	3.7(1)	0.998	4.48	−1.15(7)	2.54(9)	0.996	3.69
5	−0.90(4)	4.1(1)	0.998	5.23	−0.89(6)	2.68(9)	0.996	3.69
	V_0 (static)							
	Methanol				Acetonitrile			
	Log k'_B	A_s/n_B	r	$\mathscr{R}$	Log k'_B	A_s/n_B	r	$\mathscr{R}$
1	−1.64(7)	3.5(1)	0.997	1.03	−2.23(6)	2.87(6)	0.999	3.01
2	−1.60(7)	3.5(1)	0.997	1.09	−2.17(6)	2.84(6)	0.999	4.14
3	−1.62(7)	3.8(1)	0.997	1.05	−2.13(7)	3.01(8)	0.998	2.76
4	−1.09(5)	3.8(1)	0.998	3.70	−1.31(6)	2.67(9)	0.997	4.68
5	−1.00(3)	4.3(1)	0.999	4.67	−1.04(5)	2.80(9)	0.997	3.46

TABLE IV

CONSTANTS OF THE FUNCTIONAL RELATIONSHIP BETWEEN k' AND φ, CALCULATED BY SUBSTITUTING DATA FROM TABLES I AND II INTO EQN. 2

r = Linear regression correlation coefficient; meaning of $\mathscr{R}$ is explained in the text.

Compound	V_0							
	Methanol				Acetonitrile			
	A'	B'	r	$\mathscr{R}$	A'	B'	r	$\mathscr{R}$
1	−1.4(2)	4.5(6)	0.965	3.61	−0.7(1)	4.0(4)	0.980	9.56
2	−1.3(2)	4.1(5)	0.968	3.24	−0.7(1)	3.8(3)	0.981	17.3
3	−1.8(3)	4.8(7)	0.954	2.84	−0.8(1)	3.6(4)	0.973	12.8
4	−1.6(2)	3.5(4)	0.965	3.20	−0.7(1)	2.3(1)	0.991	6.85
5	−1.9(3)	3.6(5)	0.956	10.9	−0.7(1)	1.9(1)	0.988	5.62
	V_0 (static)							
	Methanol				Acetonitrile			
	A'	B'	r	$\mathscr{R}$	A'	B'	r	$\mathscr{R}$
1	−1.7(3)	5.3(6)	0.958	4.26	−1.0(2)	5.3(6)	0.971	6.69
2	−1.6(3)	4.8(6)	0.961	3.98	−0.9(2)	4.9(5)	0.974	9.48
3	−2.2(3)	5.8(7)	0.951	3.29	−1.0(2)	4.7(6)	0.964	6.58
4	−1.9(3)	5.0(7)	0.958	7.80	−0.9(1)	2.9(2)	0.987	11.9
5	−2.2(7)	4.3(7)	0.952	5.84	−0.9(1)	2.4(2)	0.984	5.76

TABLE V

CONSTANTS OF THE FUNCTIONAL RELATIONSHIP BETWEEN k' AND x, CALCULATED BY SUBSTITUTING DATA FROM TABLES I AND II INTO EQN. 3

r = Linear regression correlation coefficient; meaning of $\mathcal{R}$ is explained in the text.

Compound	V_0							
	Methanol				Acetonitrile			
	α	β	r	$\mathcal{R}$	α	β	r	$\mathcal{R}$
1	−1.9(3)	13(1)	0.989	1.08	−1.53(3)	36.4(2)	1.0	1.05
2	−1.7(2)	12(1)	0.987	1.03	−1.38(4)	34.0(3)	1.0	1.65
3	−2.1(2)	12(1)	0.990	1.59	−1.56(5)	27.9(3)	1.0	1.66
4	−1.3(1)	5.7(2)	0.996	2.42	−0.57(9)	9.0(4)	0.994	2.94
5	−1.4(1)	4.9(3)	0.993	5.72	−0.51(5)	5.2(2)	0.997	1.99
	V_0 (static)							
	Methanol				Acetonitrile			
	α	β	r	$\mathcal{R}$	α	β	r	$\mathcal{R}$
1	−2.4(3)	16(1)	0.987	1.25	−2.5(1)	49(1)	0.999	1.95
2	−2.2(2)	15(1)	0.988	1.16	−2.3(1)	46(1)	0.999	2.16
3	−2.7(2)	14(1)	0.983	2.06	−2.3(2)	37(1)	0.997	1.93
4	−1.7(2)	6.8(3)	0.993	3.85	−0.90(7)	11.8(3)	0.998	2.03
5	−1.8(2)	6.0(3)	0.989	11.5	−0.75(4)	6.7(3)	0.999	1.35

TABLE VI

CONSTANTS OF THE FUNCTIONAL RELATIONSHIP BETWEEN k' AND φ, CALCULATED BY SUBSTITUTING DATA FROM TABLES I AND II INTO EQN. 4

r = Linear regression correlation coefficient; meaning of $\mathcal{R}$ is explained in the text.

Compound	V_0							
	Methanol				Acetonitrile			
	Log k'_w	S	r	$\mathcal{R}$	Log k'_w	S	r	$\mathcal{R}$
1	2.4(1)	4.4(2)	0.993	1.93	2.0(1)	5.1(3)	0.990	4.26
2	2.4(1)	4.4(2)	0.992	2.01	2.0(1)	5.0(4)	0.990	5.34
3	2.7(1)	4.5(2)	0.992	1.95	2.3(1)	5.0(3)	0.989	4.66
4	2.9(1)	4.1(2)	0.995	5.84	2.1(1)	3.6(2)	0.991	7.55
5	3.3(1)	4.3(2)	0.996	7.11	2.4(1)	3.4(2)	0.993	3.98
	V_0 (static)							
	Methanol				Acetonitrile			
	Log k'_w	S	r	$\mathcal{R}$	Log k'_w	S	r	$\mathcal{R}$
1	2.5(1)	4.6(2)	0.994	1.84	2.0(1)	5.5(3)	0.990	3.82
2	2.5(1)	4.6(2)	0.993	1.79	2.0(1)	5.4(3)	0.991	4.77
3	2.7(1)	4.7(2)	0.994	1.67	2.2(1)	5.3(3)	0.990	3.41
4	3.0(1)	4.2(2)	0.996	3.33	2.1(1)	3.5(2)	0.992	5.74
5	3.4(1)	4.5(2)	0.997	6.69	2.4(1)	3.6(2)	0.994	3.91

TABLE VII

CONSTANTS OF THE FUNCTIONAL RELATIONSHIP BETWEEN k' AND φ, CALCULATED BY SUBSTITUTING DATA FROM TABLES I AND II INTO EQN. 5

r = Linear regression correlation coefficient; meaning of $\mathscr{R}$ is explained in the text.

Compound	V_0									
	Methanol					Acetonitrile				
	a	b	c	r	$\mathscr{R}$	a	b	c	r	$\mathscr{R}$
1	5.31(4)	−9.2(1)	3.47(4)	0.998	1.34	11.7(1)	−11.8(1)	2.88(4)	0.999	2.12
2	5.61(4)	−9.5(1)	3.53(4)	0.998	1.38	11.7(1)	−11.7(1)	2.88(3)	1.0	2.87
3	5.54(4)	−10.1(1)	4.01(4)	0.998	1.01	10.2(1)	−11.6(1)	3.14(4)	0.999	2.25
4	4.81(2)	−9.8(1)	4.63(3)	1.0	1.15	7.0(1)	−9.7(1)	3.45(3)	1.0	1.16
5	4.54(2)	−10.2(1)	5.21(3)	1.0	1.05	5.7(1)	−9.3(1)	3.92(3)	0.999	1.57
	V_0 (static)									
	Methanol					Acetonitrile				
	a	b	c	r	$\mathscr{R}$	a	b	c	r	$\mathscr{R}$
1	4.65(4)	−8.8(1)	3.37(4)	0.998	1.53	11.7(1)	−12.2(1)	2.92(4)	0.999	3.11
2	5.01(4)	−9.1(1)	3.44(4)	0.998	1.36	11.7(1)	−12.2(1)	2.93(3)	1.0	3.31
3	4.69(4)	−9.4(1)	3.85(4)	0.998	1.01	10.3(1)	−11.9(1)	3.17(4)	0.999	2.44
4	4.39(2)	−9.5(1)	4.52(3)	1.0	1.14	6.5(1)	−9.5(1)	3.73(3)	1.0	1.21
5	4.07(2)	−9.8(1)	5.07(3)	1.0	1.13	5.4(1)	−9.2(1)	3.85(3)	1.0	1.52

many sources of random and systematic errors make the evaluations of the weight ω_{ii} very difficult. Therefore, we arbitrarily put $\omega_{ii} = 1$ in all our calculations.

It should be noted that the numerator in eqn. 6 is the root-mean-square deviation of the fitted set, being the explicit variable in the least-squares procedure.

Different mathematical formulations of the retention model are virtually equivalent to different data sets (*i.e.*, $1/k'$, $1/k'x$, $\log k'$, φ, x) taken into account in the calculations.

It is well known that each set of experimental data can be approximated by a polynomial of sufficiently high order.

Eqns. 1–4 assume a linear relationship of the variables (a polynomial of order one) and eqn. 5 a quadratic relationship (polynomial of order two). To verify the goodness of fit of a given equation to the experimental data, the hypothesis that the approximation by the polynomial of order p-1 is as good as the approximation by the polynomial of order p was tested. Therefore, the higher power terms were added, *i.e.*, quadratic to eqns. 1–4 and cubic to eqn. 5.

The ratio

$$\mathscr{R} = R_{p-1}/R_p$$

was compared with

$$\mathscr{R}_{1,n-p-1,\alpha} = \left(\frac{1}{n-p-1}\cdot F_{1,n-p-1,\alpha} + 1\right)^{1/2} \qquad (7)$$

where F is the well known Fisher (Snedecor) distribution [42] and n is the number of experimental points. If $\mathscr{R} > \mathscr{R}_{1,n-p-1,\alpha}$, the hypothesis that the polynomial of order $p-1$ provides as good a fit as the polynomial of order p may be rejected on the $100\alpha\%$ significance level. Consequently, we may conclude that the approximation of the data by the polynomial of order $p-1$ does not give a satisfactory fit. Of course, an increase in a polynomial order always improves the agreement, and we must weigh the utility of the equation (we prefer a model with a smaller number of parameters) against the increased accuracy given by a proper choice of the significance level α. The rejection at a significance level of 1% may be regarded as "highly significant" (see ref. 41, p. 48).

The above procedure (method) seems to be particularly useful for comparison of models D and E. In these models, the same variables (log k' and φ) are used and eqn. 5 is obtained by adding a quadratic term to eqn. 4.

For the remaining models, the validation of the linear approximation was tested. The calculated values of $\mathscr{R}$ are shown in Tables III–VII; they should be compared with the values $\mathscr{R}_{1,4,0.01} = 2.51$ and $\mathscr{R}_{1,3,0.01} = 3.52$ for models A–D and E, respectively.

CONCLUSIONS

From Tables III–VII, we can see that for models A, B, D and E the differences between parameters calculated for V_0 and V_0 (static) are in most instances not greater than the sum of the corresponding standard deviations. The correlation coefficients are also very similar and values of $\mathscr{R}$ calculated for the two dead volumes lead to the same conclusions regarding the goodness of fit. This is not true for model C, for which the parameters α and β change significantly. The $\mathscr{R}$ values given in Table V (calculated for V_0) indicate that the hypothesis about the approximation of data by a straight line cannot be rejected at the 99% confidence level for four compounds (exceptions are compound **5** in methanol–water and **4** in acetonitrile–water). For V_0 (static) the $\mathscr{R}$ values are different and the hypothesis must be rejected for two compounds in methanol–water and for three in acetonitrile–water. Further considerations are based on the r and $\mathscr{R}$ values calculated for V_0 as void volume.

Table V shows that higher values of correlation coefficients are not always correlated with smaller values of $\mathscr{R}$. For example, for compound **5** in methanol–water $\mathscr{R} = 5.72$ and $r = 0.993$, but for **2**, $\mathscr{R} = 1.03$ corresponds to $r = 0.987$. Comparison of the approximation of the experimental data for these two compounds by a straight line (see Fig. 1) indicates that the $\mathscr{R}$ value is a more reliable measure of the goodness of fit. In agreement with this R value criterion, models A and D cannot be rejected only for compounds **1**–**3** in methanol–water (see Tables III and VI). For model D it is equivalent to the

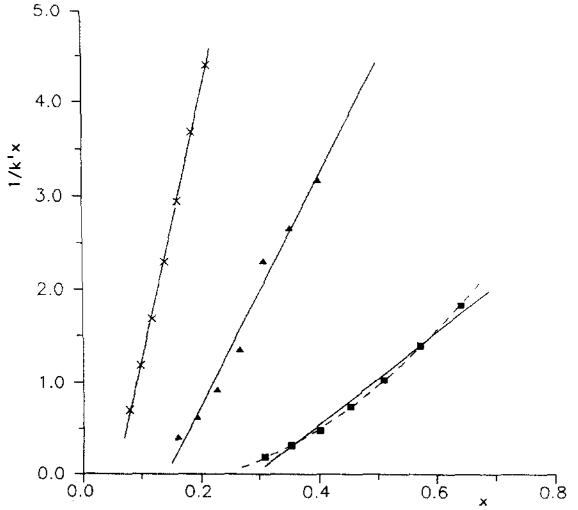

Fig. 1. Solid lines, result of fitting the experimental data to eqn. 3 (model C); dashed line, fitting the experimental data to eqn. 3 with added second-order term. × = Compound **3**, acetonitrile–water; ▲ = compound **2**, methanol–water; ■ = compound **5**, methanol–water.

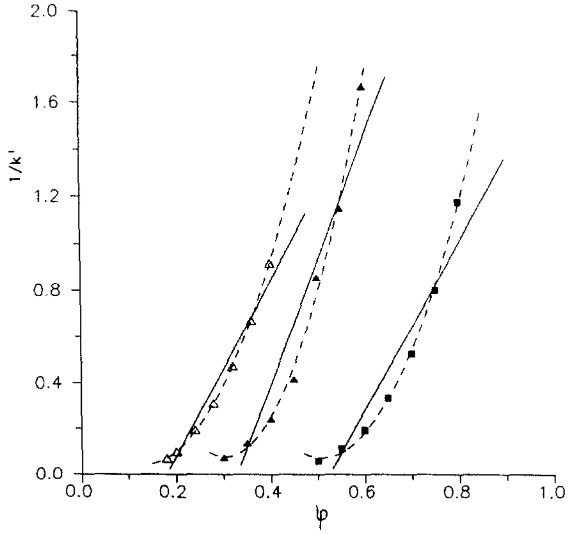

Fig. 2. Solid lines, result of fitting the experimental data to eqn. 2 (model B); dashed line, fitting the experimental data to eqn. 2 with added second-order term. △ = compound **2**, acetonitrile–water; ▲ = compound **1**, methanol–water; ■ = compound **5**, methanol–water.

conclusion that only for those data sets can we not choose between this model and model E. The latter, as can be seen from Table VII, describes satisfactorily the experimental data for all the compounds.

Model B should be rejected for all the compounds (see Table IV). Fig. 2 shows us that eqn. 2 corresponding to this model is inadequate for the approximation of our experimental data.

According to Jaffé's classification [37], the goodness of fit of the experimental data by model B is good and by models A, C, D and E is excellent even in cases when the points evidently do not follow a straight line. Hence we can conclude that the estimation of the goodness of fit based on correlation coefficients cannot be sufficiently discriminating.

It must be pointed out that our method of verification of the models does not give any indication of how to improve the equations in accordance with the physico-chemical premises of these models. Addition of a higher power term serves only for the evaluation of the goodness of fit of an original equation.

ACKNOWLEDGEMENT

This investigation was supported by a grant from the Ministry of Health and Social Welfare under MZ VII-155 to N.S.-S.

REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, Ch. 8.
2 E. Soczewiński, *Anal. Chem.*, 41 (1969) 179.
3 L. R. Snyder, *Anal. Chem.*, 46 (1974) 1384.
4 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 112 (1975) 425.
5 R. P. W. Scott, *J. Chromatogr.*, 122 (1976) 35.
6 M. Jaroniec and J. Piotrowska, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 257.
7 M. Jaroniec, J. A. Jaroniec and W. Gołkiewicz, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 89.
8 E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
9 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 32 (1968) 458.
10 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 31.
11 K. A. Dill, *J. Phys. Chem.*, 91 (1987) 1980.
12 P. Y. Ying, J. G. Dorsey and K. A. Dill, *Anal. Chem.*, 61 (1989) 2540.
13 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
14 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 218 (1981) 261.
15 R. Tijssen, H. A. H. Billiet and J. Schoenmakers, *J. Chromatogr.*, 122 (1976) 185.
16 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
17 E. Soczewiński, *J. Chromatogr.*, 388 (1987) 91.
18 X. Geng and F. E. Regnier, *J. Chromatogr.*, 296 (1984) 15.
19 J. A. Schmidt, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
20 S. R. Abbott, J. R. Berg, P. Achener and R. L. Stevenson, *J. Chromatogr.*, 126 (1976) 421.
21 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
22 K. Karch, I. Sebestian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.
23 A. Hulshoff and J. H. Perrin, *J. Chromatogr.*, 129 (1976) 263.
24 N. Tanaka and E. R. Thornton, *J. Am. Chem. Soc.*, 99 (1977) 7300.
25 J. W. Dolan, J. R. Gant and L. R. Snyder, *J. Chromatogr.*, 165 (1979) 31.
26 I. Atamna, E. Grushka, H. Colin and G. Guiochon, *Chromatographia*, 19 (1984) 48.
27 H. Engelhardt and H. Muller, *Chromatographia*, 19 (1984) 77.
28 H. A. Cooper and R. J. Hurtubise, *J. Chromatogr.*, 360 (1986) 313.
29 A. Opperhuizen, T. L. Sinnige, J. M. D. van der Steen and O. Hutzinger, *J. Chromatogr.*, 388 (1987) 51.
30 W. J. Cheong and P. W. Carr, *Anal. Chem.*, 61 (1989) 1524.
31 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
32 T. Kowalska, *Chromatographia*, 27 (1989) 628.
33 B. Neidhart, K. P. Kringe and W. Brockmann, *J. Liq. Chromatogr.*, 4 (1981) 1875.
34 G. E. Berendsen, P. J. Schoenmakers, L. de Galan, G. Vigh and Z. Varga-Puchony, *J. Liq. Chromatogr.*, 3 (1980) 1669.
35 R. J. Smith, C. S. Nieass and M. S. Wainwright, *J. Liq. Chromatogr.*, 9 (1986) 1387.
36 J. G. Dorsey and K. A. Dill, *Chem. Rev.*, 89 (1989) 331.
37 H. H. Jaffé, *Chem. Rev.*, 53 (1953) 191.
38 S. Ehrenson, R. T. C. Brownlee and R. W. Taft, *Prog. Phys. Org. Chem.*, 10 (1973) 1.
39 W. C. Hamilton, *Acta Crystallogr.*, 18 (1965) 502.
40 S. Ehrenson, *J. Org. Chem.*, 44 (1979) 1793.
41 W. C. Hamilton, *Statistics in Physical Science*, Ronald Press, New York, 1964.
42 G. W. Snedecor and W. Cochran, *Statistical Methods*, Iowa State University Press, Ames, IA, 6th ed., 1967.