



ELSEVIER

Journal of Chromatography A, 869 (2000) 57–64

JOURNAL OF  
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Adsorption/partition model of liquid chromatography for chemically bonded stationary phases of the aliphatic cyano, reversed-phase C<sub>8</sub> and reversed-phase C<sub>18</sub> types

Krzysztof Kaczmarski<sup>a,\*</sup>, Wojciech Prus<sup>b</sup>, Teresa Kowalska<sup>c</sup>

<sup>a</sup>Faculty of Chemistry, Technical University of Rzeszów, ul. W. Pola 2, 35959 Rzeszów, Poland

<sup>b</sup>Faculty of Textile Engineering and Environmental Protection, Technical University of Łódź Branch in Bielsko-Biala, Bielsko-Biala, Poland

<sup>c</sup>Institute of Chemistry, Silesian University, Katowice, Poland

## Abstract

A novel approach was introduced to modeling solute retention in the liquid chromatography systems, employing silica-based aliphatic chemically bonded stationary phases of the cyano, reversed-phase C<sub>8</sub> and reversed-phase C<sub>18</sub> types, and the mixed binary eluents most frequently used in the reversed-phase and normal-phase chromatography modes (i.e. using the methanol–water and the 2-propanol–*n*-hexane liquid mixtures, respectively). This approach takes notice of the mixed (adsorption/partition) mechanism of solute retention, in which both, the adsorptive and the dispersive forces contribute to the overall energetics of this process. Performance of our new model was compared with that of the widely recognized and on a routine basis applied Schoenmakers approach, and it was found out that both models perform with a practically equal and outstanding accuracy. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Adsorption/partition model; Partition/adsorption model; Stationary phases, LC; Mathematical modelling

## 1. Introduction

Retention of solutes in the liquid chromatography systems obviously is a very complicated process, affected by various different types of intermolecular interactions, which equally involve the molecules of solute, and of stationary and mobile phase. In order to avoid utilization of this particular separation technique simply on a trial-and-error level, and to introduce as much rationale to its practice as only possible instead, a number of the physicochemically

grounded retention models (and sometimes of the purely mathematical formulae only) have been developed. The earliest models were merely the straight-line relationships, but with the progress of modern computational techniques there is, however, no longer any need for restriction to such very simplistic approaches only and a good number of curvilinear and non-linear retention models have already been devised. One of the best performing and therefore the most widely recognized curvilinear equations was proposed by Schoenmakers [1,2]:

$$\ln k = A\varphi^2 + B\varphi + C \quad (1)$$

where  $k$  denotes the retention coefficient of solute,  $\varphi$

\*Corresponding author. Fax: +48-17-854-1260.

E-mail address: ichkk@ewa.prz.rzeszow.pl (K. Kaczmarski)

is the volume fraction of organic modifier in the mobile phase, and  $A$ ,  $B$ , and  $C$  are the equation constants. However, lacking the proper physico-chemical background, this purely mathematical model cannot explain the mechanism of the phenomena observed. On the other hand, introduction to the everyday chromatography practice of the aliphatic chemically bonded stationary phases (composed of the partially non-bonded silica matrix and of organic ligands bonded to its surface) has among the chromatography practitioners awakened an intuitive awareness of a possibility of wide occurring of the mixed (adsorption/partition) retention mechanisms, which equally involve the solute molecule's active center(s) (and hence, the adsorptive forces), and the entity of the respective molecule (through the so-called dispersive forces). It is noteworthy, that certain simple attempts of quantification of the energetic contributions from these two intermolecular interaction modes have already been accomplished (e.g. [3,4]).

Our earlier investigations of retention in the high-performance liquid chromatography (HPLC), presented in publication [5] (the chromatographic system employed: LiChrospher 100 CN as stationary phase; the methanol–water mixture of the varying volume proportions as mobile phase) demonstrated a strongly parabolic dependence between the retention coefficient,  $k$ , and the volume fraction of methanol,  $\varphi$ , for a wide number of the employed test solutes. This observation gave rise to our assumption as to the mixed (adsorption/partition) retention mechanism, involved in the process discussed. Moreover, in paper [6] it was established that these empirical results can best be approximated with aid of the Schoenmaker's retention model (which lacks any firm physico-chemical basis). Therefore in this paper a new physicochemically grounded adsorption/partition model was proposed for a novel description of the relevant experimental results. This model has also been successfully tested with use of the cyano (CN) type stationary phase/2-propanol– $n$ -hexane chromatographic system.

Examination of elution in the reversed-phase HPLC using the non-end-capped and the end-capped octyl (RP-8, RP-8e) and octadecyl (RP-18, RP-18e) stationary phases (as reported in publication [5]) also suggests that in these cases retention is of a mixed, i.e. adsorption/partition, nature. Therefore in our

present paper a further attempt is also made to apply the novel adsorption/partition model of solute retention to description of sorption on the above alkane stationary phases as well.

## 2. The adsorption/partition model of liquid chromatography

Let us consider the model of an ideal chromatographic column:

$$\frac{\partial c_1}{\partial t} + \frac{1 - \varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma}{\partial t} + w \cdot \frac{\partial c_1}{\partial x} = 0 \quad (2)$$

where  $c_1$  and  $\Gamma$  are the concentrations of the analyte in liquid phase and on the sorbent surface, respectively;  $\varepsilon_t$  is the total porosity of the solid bed;  $t$  is the time;  $x$  is the distance counted from the top of the column; and  $w$  is the real velocity of the eluent flow.

If we assume, that the process of adsorption/desorption runs with an infinitely great velocity, then the derivative of the analyte's concentration on the sorbent surface on time can be given, as follows:

$$\frac{\partial \Gamma}{\partial t} = \sum_{i=1}^n \frac{\partial \Gamma}{\partial c_i} \cdot \frac{\partial c_i}{\partial t} \quad (3)$$

where  $n$  is the number of all components of the investigated mixture (comprising equally, the components of eluent and of the chromatographed sample).

If we additionally assume, that the derivative of  $\Gamma$  on concentrations of the eluent components is negligible, then Eq. (2) can be re-written in the below given form:

$$\frac{\partial c_1}{\partial t} + \frac{w}{1 + \frac{1 - \varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma}{\partial c_1}} \cdot \frac{\partial c_1}{\partial x} = 0 \quad (4)$$

The velocity of migration of the chromatographic band is described by the expression standing before the spatial derivative of concentration. Due to the fact that the retention time,  $t_r$ , is the ratio of the column length  $H$  to the migration velocity  $w$ , we can write:

$$t_r = \frac{H}{w} \left( 1 + \frac{1 - \varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma}{\partial c_1} \right) \quad (5)$$

or

$$k = \frac{t_r - t_0}{t_0} = \frac{1 - \varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma}{\partial c_1} \quad (6)$$

where  $t_0 = H/w$ .

From widely available manufacturers' information on the chemical structure of alkyl chemically bonded stationary phases of the CN, RP-8, and RP-18 type it clearly comes out that in each such case, a considerable amount of the silica active sites (i.e. of the free silanol groups) on the non-bonded matrix surface remain free. In view of this fact it seems only sensible to assume, that the isotherm  $\Gamma(c)$  is a sum composed of the function  $\Gamma'$  (describing interactions between the components of the liquid mixture and the chemically bonded ligands) and  $\Gamma''$  (describing sorption on the free active sites of the silica matrix):

$$\Gamma = \Gamma' + \Gamma'' \quad (7)$$

For the low concentrations of the analyte, we can further assume the linear relationship between concentrations of this compound in the eluent and on the chemically bonded ligands:

$$\Gamma' = Kc_1 \quad (8)$$

and the relation given below between the constant  $K$  and the volume fraction of methanol,  $\varphi$ , as proposed in the classical Snyder's model [7]:

$$K = \exp(p'_1 + p'_2\varphi) \quad (9)$$

Function  $\Gamma''$  describes adsorption on the free active sites of silica. In the liquid–solid systems it is assumed that all the active sites are occupied either by the molecules of the analyte, or eluent, and moreover, that all the molecules compete for these active sites. Isotherm, which well accommodates the above assumptions, is the stoichiometric isotherm. Applying this isotherm to calculation of the amounts of the analyte,  $\Gamma''$ , adsorbed on the active sites of the silica, we obtain the below given equation:

$$\Gamma'' = \frac{K_1 \Gamma^\infty c_1}{K_1 c_1 + K_2 c_2 + K_3 c_3} \quad (10)$$

where  $c_1$ ,  $c_2$ , and  $c_3$  are the concentrations of the solute, methanol, and water, respectively;  $\Gamma^\infty$  is the saturation capacity of solid phase; and  $K_1$ ,  $K_2$ , and

$K_3$  are the equilibrium constants for the solute, methanol, and water, respectively.

For the low concentrations  $c_1$ , it can be written that:

$$\Gamma'' = \frac{K_1 \Gamma^\infty c_1}{K_2 c_2 + K_3 c_3} \quad (11)$$

From Eqs. (7), (8) and (11) it comes out that the sum of the derivatives of  $\Gamma'$  and  $\Gamma''$  on  $c_1$  can be given by the relationship

$$\frac{\partial \Gamma}{\partial c_1} = K + \frac{K_1 \Gamma^\infty}{K_2 c_2 + K_3 c_3} \quad (12)$$

Combining Eqs. (6), (9) and (12), we obtain:

$$k = \frac{1 - \varepsilon_t}{\varepsilon_t} \left( \exp(p'_1 + p'_2\varphi) + \frac{\Gamma^\infty K_1}{K_2 c_2 + K_3 c_3} \right) \quad (13)$$

Assuming, that the methanol–water eluent can be considered as an ideal mixture, Eq. (13) can further be given in the following form:

$$k = \exp(p_1 + p_2\varphi) + \frac{1}{p_3\varphi + p_4(1 - \varphi)} \quad (14)$$

Eq. (14) is the quintessence of the proposed adsorption/partition model of the reversed-phase chromatography employing, e.g., the methanol–water eluent.

However, in the case of the normal-phase chromatography (utilizing, e.g., the 2-propanol–*n*-hexane liquid binary mixture) it was assumed that the solutes adsorb on the chemically bonded ligands exactly in the same manner, as on the active sites of silica. In this case we can also utilize model (14) for description of the  $k=f(\varphi)$  relationship, although in a slightly modified form, i.e. dropping the first segment thereof:

$$k = \frac{1}{p_3\varphi + p_4(1 - \varphi)} \quad (14a)$$

### 3. Experimental

In order to verify the performance of Eqs. (14) and (14a), studies on solute retention by means of the HPLC were carried out with aid of the following equipment: pump: the L-6200 model Merck-Hitachi

LiChroGraph; detector: the L-4200 UV–Vis model Merck–Hitachi LiChroGraph; integrator: the D-7500 model Merck–Hitachi; Merck column thermostat; and the 5  $\mu$ l injection loop.

Experimental conditions: the mobile phase flow rate was 1 ml/min; absorbance was measured at the column's end at 230 nm; and column temperature was 30°C.

As mobile phases we used the following liquid binary mixtures:

1. Methanol–water (the chromatographic grade LiChrosolv Merck methanol, and water demineralized in the Millipore system and then degassed in ultrasonic bath); this eluent in chang-

ing volume proportions was utilized in the cases of the CN, RP-8, and RP-18 type stationary phases.

2. 2-Propanol–*n*-hexane (the chromatographic grade LiChrosolv Merck 2-propanol and *n*-hexane); this eluent in changing volume proportions was utilized in the case of the CN type stationary phase only.

Basic characteristics of the applied stationary phases in form of the ready-made HPLC columns are given in Table 1.

Test solutes employed in our experiment and the origins of the respective samples are given in Table 2.

Table 1

Basic characteristics of the applied stationary phases in form of the ready-made columns (119 mm  $\times$  4 mm I.D.), produced by the firm E. Merck, Germany

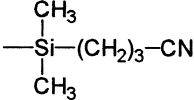
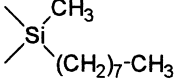
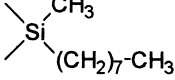
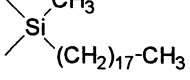
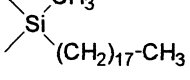
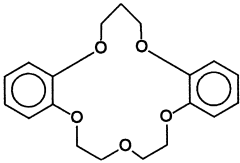
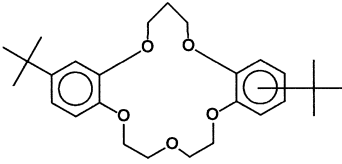
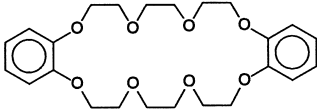
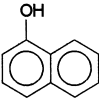
Stationary phase	Cat. No.	Chemically bonded ligand	'Endcapping'	Carbon content (%)	Density of coverage $\Theta$ ( $\mu$ mol/m <sup>2</sup> )	Dead time (min) $t_R$ (NO <sub>3</sub> <sup>-</sup> )
LiChrospher 100 CN (5 $\mu$ m)	50825		None	6.6	3.25	0.526 (RP) 1.313 (NP)
LiChrospher 100 RP-8 (5 $\mu$ m)	50822		None	12.5	4.04	0.673
LiChrospher 100	50827		-Si(CH <sub>3</sub> ) <sub>3</sub>	13.0	4.44	0.746
RP-8 endcapped (5 $\mu$ m) LiChrospher 100	50823		None	21.0	3.61	0.586
RP-18 (5 $\mu$ m) LiChrospher 100 RP-18 endcapped (5 $\mu$ m)	50828		-Si(CH <sub>3</sub> ) <sub>3</sub>	21.6	4.09	0.773

Table 2  
Test solutes

	Dibenzo-16-crown-5 obtained as described in [8]
	4,4'(5')-Di- <i>tert.</i> -butyl- dibenzo-16-crown-5 obtained, as described in [8]
	Dibenzo-24-crown-8 obtained as described in [8]
	1-Naphthol, analytical grade, Fluka, Switzerland cat. No. 70438

## 4. Verification of the model

### 4.1. The CN type stationary phase

#### 4.1.1. The reversed-phase chromatography; eluent: methanol–water

In Figs. 1 and 2 we showed the results of our experiment for the two different crown ethers, which demonstrate a strongly parabolic  $k = f(\varphi)$  relationship. On the same figures the theoretical curves are plotted, calculated with aid of Eq. (14). In Table 3 we presented the results of estimation of the model's parameters. These estimation was carried out through minimization of a sum of the squared differences between the experimental and the theoretical data, using the Marquardt method [9]. The accuracy of determination of the model's parameters was calcu-

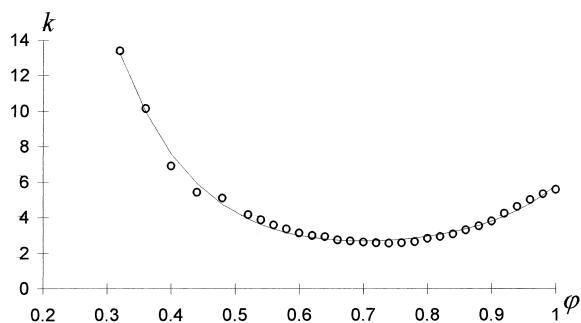


Fig. 1. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. The CN type stationary phase; the test solute: dibenzo-16-crown-5.

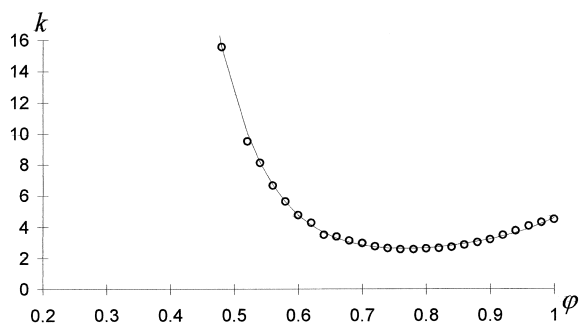


Fig. 2. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. The CN type stationary phase; the test solute: 4,4'(5')-di-*tert.*-butyldibenzo-16-crown-5.

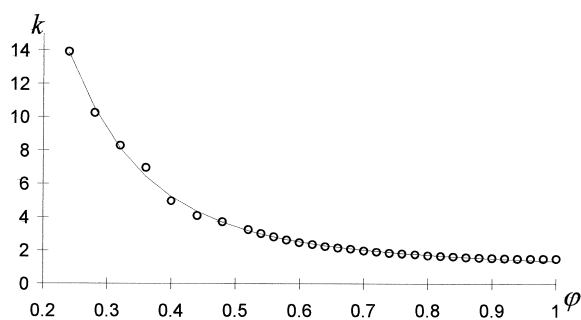


Fig. 3. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. The CN type stationary phase; the test solute: 1-naphthol.

lated upon the formulas given in the monograph [10] for the 95% confidence interval of Student's  $t$ -test.

In Table 3 we also compared the sums of the squared differences between the experimental and the theoretical data, calculated with aid of model (14) ( $S1$ ) and of the widely recognized Schoenmaker's model ( $S2$ ). As it comes out from this comparison, model (14) very well describes the experimental results and therefore its performance cannot be regarded as a slightest worse than that of the Schoenmaker's model. With the analytes less polar than the crown ethers discussed in this paper, no parabolic  $k = f(\varphi)$  relationship was observed, but with these less polar analytes the performance of model (14) is also fully analogous to that of the Schoenmaker's model. In Fig. 3 the relevant experimental illustration is presented, and in Table 3 the estimated model parameters are given. With the less polar solutes the best theoretical description was obtained, when assuming  $p_4$  as equal to zero ( $p_4 = 0$ ) (i.e. when assuming, that intermolecular interactions with participation of water ought to be excluded from our considerations).

#### 4.1.2. The normal-phase chromatography; eluent: 2-propanol-*n*-hexane

In the case of the normal-phase chromatography, calculations were performed with aid of a somewhat simplified Eq. (14a). This relationship especially well performs for the solutes with the strongly pronounced ability to adsorb. An example of practical verification of Eq. (14a) is given in Fig. 4, and the

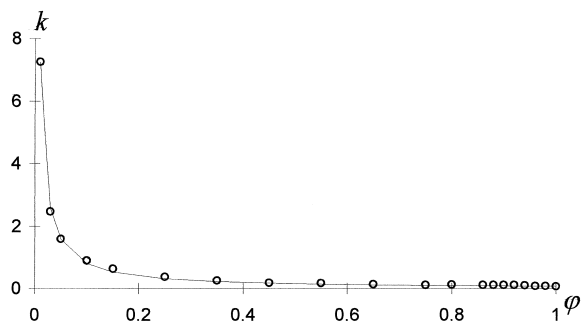


Fig. 4. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of 2-propanol. The CN type stationary phase; the test solute: 1-naphthol.

Table 3

The estimated numerical values of the parameters of model (14) and the sums of the squared differences between the experimental and the theoretical data, calculated with aid of model (14) ( $S1$ ) and of the Schoenmaker's model ( $S2$ )

Fig.	$p_1$	$p_2$	$p_3$	$p_4$	$S1$	$S2$
1	$5.08 \pm 0.207$	$-8.10 \pm 0.599$	$0.173 \pm 0.0106$	$1.16 \pm 0.144$	1.55	1.17
2	$8.70 \pm 0.233$	$-12.6 \pm 0.473$	$0.219 \pm 9.33 \cdot 10^{-3}$	$1.20 \pm 0.111$	0.408	1.33
3	$4.33 \pm 0.156$	$-9.23 \pm 0.650$	$0.748 \pm 0.0334$	0	0.580	1.21
4	—	—	$12.5 \pm 0.556$	$0.0133 \pm 0.00617$		

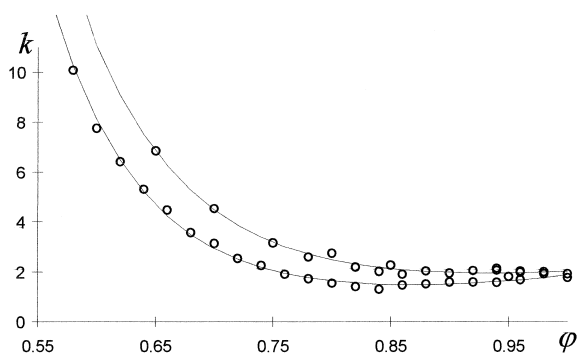


Fig. 5. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. Solute: dibenzo-24-crown-8. The upper line: RP-18; the bottom line: RP-18e.

respective numerical values of the model parameters are given in Table 3.

#### 4.2. The RP-8 and RP-18 type stationary phases

In Figs 5–8 we gave the examples of the experimentally measured retention data, and also the plots calculated with the aid of Eq. (14). In Table 4 the results are given, dealing with estimation of the parameters of this model. Again, estimation was carried out through minimization of a sum of the squared differences between the experimental and the theoretical data, using the Marquardt method [9]. The accuracy of determination of the model's parameters was calculated upon the formulas given in the monograph [10] for the 95% confidence interval of

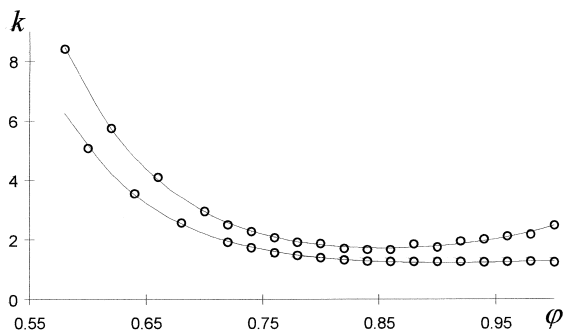


Fig. 6. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. Solute: dibenzo-24-crown-8. The upper line: RP-8; the bottom line: RP-8e.

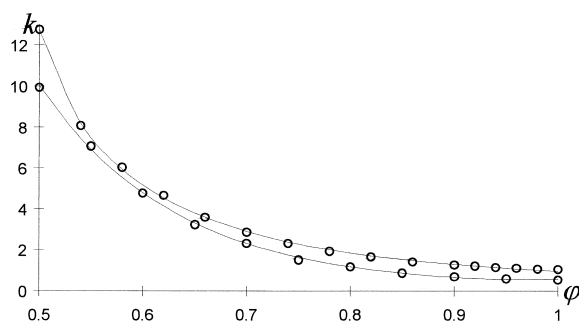


Fig. 7. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. Solute: 1-naphthol. The upper line: RP-18; the bottom line: RP-18e.

Student's  $t$ -test. In Table 4 we also presented the sums of the squared differences between the experimental data and the theoretical ones [calculated either according to model (14) (S1), or to the Schoenmaker's model [1,2] (S2)]. Plots given in Figs. 5–8 are very typical of all these obtained for several dozens of the examined test solutes. The retention coefficients ( $k$ ) (and simultaneously the observed retention times) are always lower for the end-capped packings, when compared with their respective non-end-capped counterparts. This phenomenon can be explained in the following way: An additional coverage of the silica surface with dimethylsilyl groups eliminates certain part of the silanol active sites on the silica surface, which are not bonded with the octyl (RP-8), or octadecyl (RP-18) ligands. In the most cases, the best fit of the experimental data to model (14) is observed with the

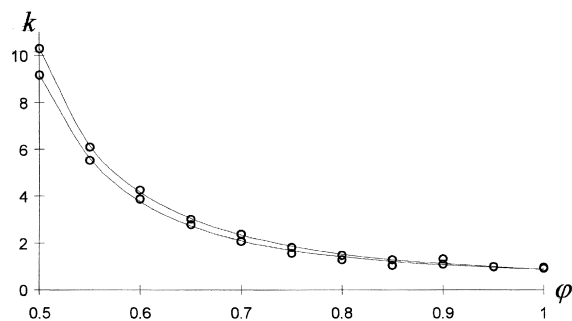


Fig. 8. Dependence of the retention coefficient,  $k$ , on the volume fraction,  $\varphi$ , of methanol. Solute: 1-naphthol. The upper line: RP-8; the bottom line: RP-8e.

Table 4

The estimated numerical values of the parameters of model (14) and the sums of the squared differences between the experimental and theoretical data for model (14) ( $S1$ ) and for the Schoenmaker's model ( $S2$ ). In the first line of a given verse the results are given for the end-capped sorbent, and in the second line for the sorbent without the end-capping

Fig.	Sorbent	$p_1$	$p_2$	$p_3$	$p_4$	$S1$	$S2$
5	RP-18e	$9.68 \pm 0.435$	$-12.8 \pm 0.777$	$0.542 \pm 0.0663$	$2.47 \pm 0.923$	0.471	0.413
	RP-18	$9.06 \pm 1.80$	$-11.3 \pm 2.91$	$0.521 \pm 0.0482$	$1.36 \pm 1.32$	0.255	0.289
6	RP-8e	$8.59 \pm 0.349$	$-11.9 \pm 0.604$	$0.789 \pm 0.0207$	$1.81 \pm 0.335$	0.0096	0.057
	RP-8	$8.50 \pm 0.292$	$-11.1 \pm 0.502$	$0.413 \pm 0.0173$	$2.63 \pm 0.363$	0.0631	0.144
7	RP-18e	$6.20 \pm 0.291$	$-7.91 \pm 0.617$	$3.68 \pm 2.37$	0	0.088	0.132
	RP-18	$7.88 \pm 0.535$	$-11.1 \pm 1.07$	$0.907 \pm 0.126$	0	0.576	0.570
8	RP-8e	$7.89 \pm 0.667$	$-11.8 \pm 1.35$	$1.08 \pm 0.164$	0	0.145	0.162
	RP-8	$7.98 \pm 0.584$	$-11.8 \pm 1.19$	$1.02 \pm 0.146$	0	0.145	0.180

parameter  $p_4=0$ . Only in the case of dibenzo-24-crown-8, used as a test solute (and particularly in the case of the RP-8e packing), the volume fraction of water in the mixed eluent markedly affects our relationship of  $k=f(\varphi)$ . In this particular case it also happens that the parabolic  $k=f(\varphi)$  dependence is observed. From a comparison of the results given in Table 4 (i.e. of the values of the sums of the squared differences between the experimental and theoretical results) once more it comes out that the performance of model (14) is fully equivalent to that of the Schoenmaker's model.

## 5. Conclusions

In this paper a new adsorption/partition model was proposed for description of the retention coefficient,  $k$ , of a given solute as a function of the mixed mobile phase composition. This model was thoroughly tested in the experiments with use of (i) a column packed with 3-cyanopropyl ligands chemically bonded to the silica matrix (both in the normal and the reversed chromatography systems), and also of (ii) columns packed with the non-end-capped and end-capped RP-8 and RP-18 stationary phases.

All the computational results obtained confirm very good performance of model (14) (and also of its minor (14a) modification). These results seem to convincingly point out to the dual nature of the retention process: On the one hand, the solute's molecules tend to interact with the organic ligands of stationary phase through the dispersive forces (the partition mode), and on the other hand, they compete

with the mobile phase molecules to active sites on the surface of the inorganic matrix (the adsorption mode). This hypothesis is particularly well confirmed by the relatively lower numerical values of the coefficient  $k$ , obtained by all the investigated solutes on the end-capped RP-8 and RP-18 stationary phases (as compared with the respective non-end-capped sorbents).

In order to scrutinize the proposed adsorption/partition model even better, one needs to perform an additional experimental study with the concentration overload of the column (which is going to be the next step of the authors' further research).

## References

- [1] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, L. de Galan, J. Chromatogr. 149 (1978) 519.
- [2] P.J. Schoenmakers, Optimization of Chromatographic Selectivity — A Guide to Method Development (Journal of Chromatography Library, 35), Elsevier, Amsterdam, 1986.
- [3] T. Kowalska, B. Witkowska-Kita, J. Planar Chromatogr. 9 (1996) 92.
- [4] B. Witkowska-Kita, T. Kowalska, J. Planar Chromatogr. 9 (1996) 368.
- [5] W. Prus, Ph.D. Thesis, Silesian University, Katowice, 1997
- [6] W. Prus, Y. Vander Heyden, P. Vankeerberghen, P. Kuś, D.L. Massart, T. Kowalska, Chemomet. Intell. Lab. Syst. (in press)
- [7] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. 165 (1979) 3.
- [8] P. Kuś, J. Planar Chromatogr. 8 (1995) 472.
- [9] D.W. Marquardt, J. Appl. Math. 11 (1963) 431.
- [10] G.A.F. Seber, C.J. Wild, Nonlinear Regression, Wiley, New York, 1989.