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Modeling of the influence of the modifier concentration on the retention process in NP-HPLC

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The adsorption model for an accurate prediction of the analyte retention in the normal – phase liquid chromatography with a binary mobile phase has been proposed. This model was derived using a thermodynamically consistent modified competitive Langmuir isotherm. The performance of the proposed equation was compared with two retention models reported in literature. All models were verified for different NP-LC systems by use of three criteria: the sum of squared differences between the experimental and theoretical data, approximation of the standard deviation and the Fisher test.

Keywords: NP-HPLC, retention models, mobile phase composition.

INTRODUCTION

The designing of industrial chromatographic separation processes requires an efficient optimization tool, which can be used to determine the optimum operating conditions rapidly and robustly. One of the most important variables typically used for the optimization of chromatography processes is the mobile phase composition, i.e. the concentration of the mobile phase modifier.

In a liquid chromatography the composition of the mobile phase determines the retention time of solutes. The composition modifications and the nature of the mobile phases enable both the tuning of the separated analytes' retention in a wide range of the retention parameters and the optimization of the chromatographic processes as well. The optimization of separation selectivity can be achieved by several different methods. One of them is the so-called interpretative strategy[1]. The key role in this strategy is the implementation of an adequate model of retention that couples the retention of a solute with the composition of a mixed eluent. This model should preferably have a sound physicochemical basis. In the liquid – solid (i.e. liquid chromatography) systems, the major role is played by the intermolecular solute – stationary phase interactions. In such systems it is assumed that all the active sites of the stationary phase are occupied by the molecules of the analyte or the eluent compounds and moreover that all the molecules compete for these active sites. From this point of view, it was justifiably assumed that the presented above mechanism may be important in the prediction of retention in a normal phase liquid chromatography. For this reason, in this paper a new adsorption model is proposed, valid particularly for the normal-phase liquid chromatography. This model was derived on the base of a thermodynamically consistent modified competitive Langmuir-like isotherm. The proposed model was examined with the use of different NP-HPLC systems. Moreover, the aim of this work was to analyse the accuracy and applicability of the proposed model in comparison with the two most popular retention models for NP-HPLC taken from literature. Despite the fact that there are many models available, investigations to find more precise models are still being performed, and it was also the subject of this work.

THE RETENTION MODEL OF NP-HPLC

Let us consider the model of an ideal chromatographic column[2]:

$$\frac{\partial c_i}{\partial t} + \frac{1-\varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma_i}{\partial t} + w \cdot \frac{\partial c_i}{\partial x} = 0 \tag{1}$$

where: c_i and Γ_i are the concentrations of i-th component in the liquid phase and on the sorbent surface, respectively; ε_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 linear eluent flow velocity.

If we assume that the adsorption/desorption process is infinitely fast and the concentration of the analyte, c_1, is very low in comparison with the modifier and the main component of the eluent concentrations (c_2 and c_3 respectivelly), then the changes of the concentration c_1 cause practically negligible perturbations of the eluent's components concentrations c_2 and c_3. Thus, the time derivative of the surface analyte concentration can be approximated as follows:

$$\frac{\partial \Gamma_i}{\partial t} = \sum_{i=1}^{3} \frac{\partial \Gamma_i}{\partial c_i} \cdot \frac{\partial c_i}{\partial t} \cong \frac{\partial \Gamma_1}{\partial c_1} \cdot \frac{\partial c_1}{\partial t} \tag{2}$$

Therefore, the eq. (1), with reference to the analyte only, can be re-written as follows:

$$\frac{\partial c_1}{\partial t} + \frac{w}{1 + \frac{1-\varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma_1}{\partial c_1}} \cdot \frac{\partial c_1}{\partial x} = 0 \tag{3}$$

where: c_1 and Γ_1 are the concentrations of the analyte in the liquid phase and on the adsorbent surface, respectively.

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

$$t_r = \frac{H}{w} \cdot \left(1 + \frac{1-\varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma_1}{\partial c_1}\right) \tag{4}$$

or after the transformation of eq. (4), the retention factor of analyte, k, can be described as follows:

$$k = \frac{t_r - t_0}{t_0} = \frac{1-\varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma_1}{\partial c_1}, \quad \text{where: } t_0 = \frac{H}{w} \tag{5}$$

In the multicomponent chromatographic systems, we can account for the competitive behavior of three components using a modified competitive Langmuir isotherm[3]. For the analyte this isotherm can be written as follows:

$$\Gamma_1 = \frac{\left(\Gamma_1^\infty - \Gamma_2^\infty\right)K_1c_1}{1+K_1c_1} + \frac{\left(\Gamma_2^\infty - \Gamma_3^\infty\right)K_1c_1}{1+K_1c_1+K_2c_2} + \frac{\Gamma_3^\infty K_1c_1}{1+K_1c_1+K_2c_2+K_2c_2} \tag{6}$$

where: c_i are the concentrations of the analyte and the mobile phase compounds, Γ_i^∞ are the saturation capacities of the analyte and the mobile phase compounds; and K_i are the equilibrium constants of the chromatographic system compounds.

It should be noticed that the eq. (6) is thermodynamically consistent – this isotherm in opposite to the classical competitive Langmuir or Langmuir-like isotherm models does not assume equal saturation capacities for different components of the chromatographic system[3]. The first term on the right side of eq. (6) is the expression for the amount of component 1 that adsorbs without any competition. The second term represents the amount of component 1 adsorbed on the surface in competition with component 2. The third term of eq. (6) represents the amount of component 1 adsorbed on the surface in competition with components 2 and 3.

The modified competitive Langmuir isotherm eq. (6) has been applied in eq. (5) for the derivation of the retention model.

In the case of low analyte concentrations, c_1, (analytical mode: $c_1 \to 0$), equation (6) can be written as follows:

$$\Gamma_1 = \left(\Gamma_1^\infty - \Gamma_2^\infty\right)K_1c_1 + \frac{\left(\Gamma_2^\infty - \Gamma_3^\infty\right)K_1c_1}{1+K_2c_2} + \frac{\Gamma_3^\infty K_1c_1}{1+K_2c_2+K_2c_2} \tag{7}$$

The derivative of Γ_1 on c_1 gives the following relation:

$$\frac{\partial\Gamma_1}{\partial c_1} = \left(\Gamma_1^\infty - \Gamma_2^\infty\right)K_1 + \frac{\left(\Gamma_2^\infty - \Gamma_3^\infty\right)K_1}{1+K_2c_2} + \frac{\Gamma_3^\infty K_1}{1+K_2c_2+K_2c_2} \tag{8}$$

Combining equations (5) and (8) we obtain:

$$k = \frac{1-\varepsilon_t}{\varepsilon_t}\cdot\left(\left(\Gamma_1^\infty - \Gamma_2^\infty\right)K_1 + \frac{\left(\Gamma_2^\infty - \Gamma_3^\infty\right)K_1}{1+K_2c_2} + \frac{\Gamma_3^\infty K_1}{1+K_2c_2+K_2c_2}\right) \tag{9}$$

After the conversion of the mobile phase compounds' molar concentrations into the molar fractions, equation (9) can further be given in the following form:

$$k = p'_1 + \frac{p'_2}{1+p'_3\varphi} + \frac{p'_4}{1+p'_3\varphi+p'_5(1-\varphi)} \tag{10}$$

After simple mathematical transformations the final relationship between the retention coefficient, k, and the modifier concentration in the binary mobile phase, φ, takes the following form:

$$k = \frac{p_1 + \dfrac{p_2\cdot(1-\varphi)}{1+p_3\cdot\varphi} + p_4\cdot\varphi}{1+p_3\cdot\varphi+p_5\cdot(1-\varphi)} \tag{11}$$

where: p_i are the experimental equation parameters. Note, that only positive values of the parameters should be taken into consideration.

The aim of this work was to analyse the accuracy of the presented model eq. (11) for the description of retention processes in different NP-HPLC systems. For the experimental verification of the proposed model eq. (11) the literature data[4–7] from NP-HPLC measurements were used. Table 1 specifies the example samples, mobile phases, the range of the modifier volume or mole fractions, and the type of chromatographic columns used.

The equation constants (p_i) were estimated by minimization of a sum of the squared differences between the experimental and theoretical data using the Marquardt method[8]. The accuracy of determination of the model's parameters was assessed for the 95% confidence interval of Student's test. The

Table 1. Test analytes, mobile phases, ranges of the modifier volume or mole fractions and the HPLC columns used

Set	Test analyte	Mobile phase	Range of modifier vol. or mole fraction	Column
1	4,4'(5')-di-tert-butyldibenzo14-crown-4	2-propanol – n-hexane	0.01 – 1.0 (2-propanol)	LiChrospher 100 CN 125x4 mm, 5μm Merck, Darmstadt Germany
2	1-Naphthol		0.01 – 1.0 (2-propanol)	
3	p-cresol		0.0 – 1.0 (2-propanol)	
4	2,3-dimethylphenol		0.0 – 1.0 (2-propanol)	
5	2-methylquinoline		0.05 – 1.0 (2-propanol)	
6	6-methylquinoline		0.05 – 1.0 (2-propanol)	
7	8-methylquinoline		0.0 – 1.0 (2-propanol)	
8	8-hydroxy-2-methylquinoline		0.05 – 1.0 (2-propanol)	
9	Ethyl acetate	pentane – diethyl ether	0.0 – 0.5 (diethyl ether)	Hypersil Cyanopropyl 250x4.6 mm, 5μm Shandon, Eragny France
10	Butyl isothiocyanate		0.0 – 0.5 (diethyl ether)	LiChrospher 100 Diol 250x4.6 mm, 5μm Shandon, Eragny France
11	Ethyl acetate		0.0 – 0.5 (diethyl ether)	
12	Ethyl(E)-2-butenoate		0.0 – 0.5 (diethyl ether)	
13	Triphenylene		0.0 – 0.5 (diethyl ether)	
14	Butyl isothiocyanate		0.0 – 0.5 (diethyl ether)	Hypersil Aminopropyl 250x4.6 mm, 5μm Shandon, Eragny France
15	Ethyl acetate		0.0 – 0.5 (diethyl ether)	
16	Ethyl(E)-2-butenoate		0.0 – 0.5 (diethyl ether)	
17	Triphenylene		0.0 – 0.5 (diethyl ether)	
18	Phenanthrene		0.0 – 0.5 (diethyl ether)	
19	3-(4-isopropylphenyl)-1,1-dimethylurea	2-propanol – n-heptane	0.03 – 0.3 (2-propanol)	Silica gel Separon SGX 150x3.3 mm, 7μmTessek, Czech Republic
20	3-(3-chloro-p-tolyl)-1,1-dimethylurea	dioxane – n-heptane	0.1 – 0.5 (dioxane)	
21	3-(4-isopropylphenyl)-1,1-dimethylurea		0.1 – 0.5 (dioxane)	
22	3-(4-chlorophenyl)-1,1-dimethylurea		0.1 – 0.5 (dioxane)	
23	1-butyl-3-(3,4-dichlorophenyl)1-methylurea		0.05 – 0.5 (dioxane)	

Table 2. The values of the estimated model parameters pi (equations (11) and (15 – 16)) and the Fisher test values

set	Eq. (11)					F	Eq. (15)		F	Eq. (16)		F
	p_1	P_2	p_3	p_4	p_5		p_1	p_2		p_1	p_2	
1	4.75±0.5	0.424±0.1	32.61±4.2	12.8±9.1	6.24e-7±3e-8	275.4	4.09±0.9	0.23±0.04	23.1	-0.67±0.09	0.4±0.02	158.4
2	43.9±4.4	28.99±10.1	568.8±37	18.9±3.8	0.0±0.0	1359.0	12.5±0.6	0.01±6e-3	1133	-2.36±0.07	0.9±0.02	2147
3	34.5±0.1	144.6±1.2	511.8±22	4.1e-6±1e-7	8.77±0.7	2.34e4	19.2±0.8	0.06±4e-4	2902	-2.35±1.1	0.81±2.6	330.1
4	18.6±1.2	37.7±4.7	307.6±22	1.9e-7±5e-8	5.43±0.4	9687.0	21.2±1.1	0.11±0.01	1061	-2.36±0.7	0.75±0.6	193.2
5	1.3e-7±1e-8	19.14±8.5	12.66±10	2.75±1.6	7.24±3.4	154.6	7.52±0.7	0.40±0.07	44.9	-1.79±0.1	0.7±0.04	53.86
6	3.9e-7±1e-8	21.98±10.7	12.77±3.0	2.99±1.0	4.61±2.2	98.54	5.91±0.5	0.17±0.04	65.0	-1.63±0.1	0.8±0.02	52.25
7	4e-8±1e-9	2.14±1.0	4.004±2.1	0.84±0.5	3.08±1.6	65.59	8.90±1.1	2.06±0.2	9.9	-1.95±0.1	0.4±0.04	18.78
8	1.85±0.3	26.35±2.6	4.59±0.9	1.2e-6±2e-7	5.08±1.5	97.91	1.82±0.2	0.18±0.03	28.9	-0.43±0.2	0.6±0.09	11.14
9	7.2±0.1	11.48±0.7	93.89±6.1	1.0e-6±4e-7	38.05±2.07	2.91e4	34.1±4.5	2.31±0.8	7.1	-2.34±0.2	0.3±0.04	279.7
10	5.5±0.0	7.06±0.0	65.2±0.0	2.334±0.0	22.41±0.0	6.2e8	19.9±10.	2.05±0.6	7.2	-2.01±0.1	0.2±0.04	305.8
11	54.02±0.0	94.04±0.0	1060.0±0	62.59±0.0	66.68±0.0	2.5e8	69.8±7.9	0.46±0.1	16.2	-1.90±0.8	0.36±0.2	36.0
12	66.0±0.01	76.2±6.7	1605.0±18	128.6±39.7	62.51±2.9	3.50e4	58.5±39.	0.45±0.12	19.6	-1.95±0.8	0.39±0.2	50.1
13	43.8±13.1	48.2±28.2	156.4±59	5.1e-6±3e-7	29.07±1.8	4339.0	6.71±4.5	0.36±0.08	12.0	-0.67±0.4	0.29±0.1	25.63
14	19.02±0.0	23.1±0.0	695.2±0.0	37.73±0.0	61.26±0.0	1.86e8	88.95±15	1.58±0.8	5.4	-2.37±0.5	0.27±0.1	39.33
15	65.4±0.01	88.96±5.2	3975±78	283.0±78.8	60.45±5.0	5.96e4	104.9±64	0.39±0.06	81.9	-2.66±0.1	0.5±0.02	9690
16	21.7±4.3	221.9±89.0	371.0±38	1.04e-6±8e-7	91.62±35.1	2.47e4	98.04±54	0.38±0.05	102	-2.72±0.1	0.52±0.3	4489
17	97.5±8.2	638.5±87.5	529.4±41	2.5e-5±9e-6	110.9±14.1	4.8e4	39.4±29	0.15±0.03	33.6	-1.32±0.2	0.41±0.1	232.8
18	41.2±1.7	166.7±9.8	385.0±44.	1.02e-6±1e-7	101.0±34.1	3.84e4	70.4±50.	0.49±0.1	12.1	-1.88±0.2	0.3±0.04	183.0
19	89.4±3.1	6117.0±64.4	245.3±27	1.84e-6±2e-7	11.71±2.8	6472.0	0.84±0.3	2.66e-8±0.0	42.5	-0.81±0.2	1.3±0.07	2170
20	17.1±2.04	2937.0±79.3	84.0±11.3	1.68±0.2	1.78±0.5	7.47e5	0.49±0.2	4.97e-7±6e-8	5.4	-0.95±0.2	1.8±0.09	2740
21	51.2±3.8	1.45e4±221	188.7±14	3.24e-7±6e-8	2.884±0.4	2.03e5	0.46±0.1	3.7e-6±4e-7	4.1	-0.95±0.1	1.9±0.05	8736
22	53.98±3.4	3.79e4±510	274.7±18	17.76±1.52	8.44±0.8	1.14e7	0.45±0.1	5.2e-6±9e-7	3.3	-0.83±0.2	1.9±0.08	3630
23	3.61±0.3	45.2±9.8	20.44±6.4	1.53e-61e-7±	0.051±0.001	2939.0	1.69±0.8	4.8e-8±1e-8	29.6	-1.23±0.4	1.2±0.1	296.6

following statistical criteria were used for the assessment of the proposed model accuracy in different HPLC systems:

– The sum of squared differences between the experimental and the theoretical retention data:

$$SUM = \sum_i \left(k_{exp}(i) - k_{theor}(i)\right)^2 \tag{12}$$

– Approximation of the standard deviation:

$$SD = \sqrt{\frac{SUM}{N-l}} \tag{13}$$

– The Fisher test:

$$F = \frac{(N-l)\cdot \sum_{i=1}^{N}\left(k_{\exp}(i) - \sum_i \frac{k_{\exp}(i)}{N}\right)^2}{(N-1)\cdot \sum_{i=1}^{N}\left(k_{\exp}(i) - k_{theor}(i)\right)^2} \tag{14}$$

where: $i = 1 \ldots N$, N – number of experimental points, l – number of estimated model parameters.

RESULTS AND DISCUSSION

Validation of the proposed model in different chromatographic systems

Table 2 specifies the values of the estimated model parameters pi and the Fisher test values obtained as the result of comparison between the proposed model (eq. (11)) and the experimental data. The related sums of the squared differences between experimental and theoretical data, and SDs are presented in Figs. (1 – 2). In Fig. (3) the example graphical comparisons of experimental retention values (k) of solutes with the theoretical data have also been placed.

On the basis of comparisons between the theoretical and the experimental data presented in Table 2 and in Figs. (1 – 3) it can be concluded that the five – parameter model proposed in this study (eq. (11)) provides an excellent agreement between the experimental and the theoretical data for most NP chromatographic systems studied. The related values of SUMs and SDs are very low (see Figs. (1 – 2)) and the values of Fisher test are in many cases larger than 10^4 (see Table 2).

Comparison of proposed model eq. (11) with other retention models of NP-HPLC

The second purpose of this work was the comparison of the proposed model (eq. (11)) with the two literature-known retention models:

– the adsorption model proposed by Kaczmarski and coworkers[9]. This model was derived using a thermodynamically inconsistent three – component stoichiometric isotherm:

$$k = \frac{1}{p_1 \cdot \varphi + p_2 \cdot (1-\varphi)} \tag{15}$$

– the retention model derived from the Snyder-Soczewiński theory that assumes the monolayer adsorption of a polar component of the eluent on the adsorbent surface and their displacement by the molecules of the chromatographed compounds[10 – 11]:

$$\ln k = p_1 - p_2 \cdot \ln\varphi \tag{16}$$

All the models were compared in different NP-HPLC systems presented in Table 1, by means of SUM and SD as statistical criteria (eqs. (12) – (13)) – see Figs. (1 – 2). Besides, all models tested in this work that have different numbers of parameters, were statistically compared with the use of Fisher test (eq. (14)) – see Table 2. The best model is the one that exhibits the highest value of the Fisher parameter[12]. As clearly seen in eq. (14), the fact that the two models may have different numbers of adjustable parameters is ac-

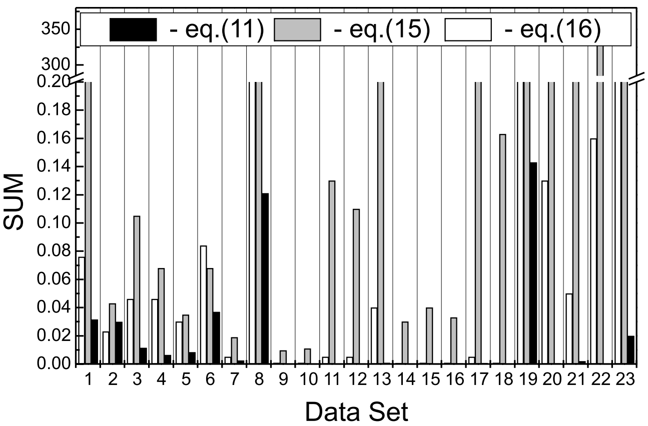

Figure 1. A graphical comparison of SUMs for the analysed models and data sets (see Table 1)

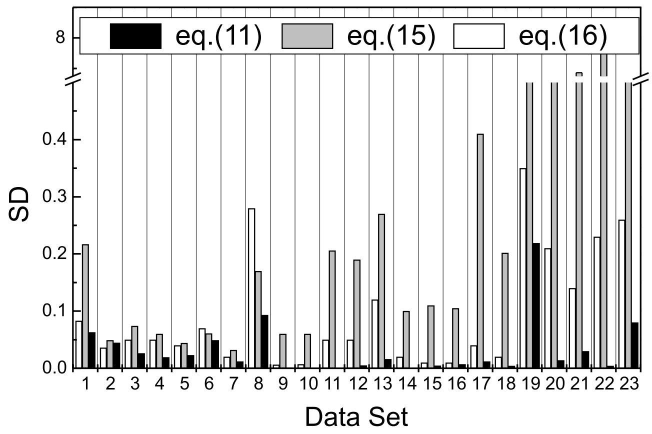

Figure 2. A graphical comparison of SDs for the analysed models and data sets (see Table 1)

counted for in the definition of F[12] (everything else being equal, F decreases with the increasing number of the parameters of the tested model). Thus, the Fisher test, F, is a convenient assessment parameter enabling the comparison of the different models with regard to the accuracy of the experimental data[12].

In Figs. (1 – 2), the values of the sums of squared differences between the experimental and the theoretical data, and the SDs for all models tested in this work were compared. Table 2 specifies also the values of the estimated model parameters and the Fisher test values obtained as a result of the comparison between the equations (15 – 16) and the experimental data. In Fig. 3 dashed lines show the theoretical curves obtained from eqs. (15 – 16). From the comparison of the three statistical parameters (see Figs. (1 – 2) and Table 2) one can see that eq. (11) significantly better describes the experimental data tested in this work (gives lower values of SUMs and SDs and higher values of Fisher tests) than equations (15 – 16). It is necessary to notice, that the proposed eq. (11) more precisely describes $k=f(\varphi)$ relations in comparison with eq. (15), which was formulated on the basis of a thermodynamically inconsistent stoichiometric isotherm. Recapitulating, in the case of NP-HPLC systems, taking into account all statistical criteria, the proposed thermodynamically consistent model (eq. (11)) quantitatively describes the analysed process very well and is evidently better than other models tested in this work.

SUMMARY

– The quantitative retention versus the eluent composition relationships have a fundamental significance for the method development in chromatography. Therefore, in this study an adsorption equation was proposed for the description of the retention coefficient, k, of a given solute as a function of the mixed mobile phase composition.

– The proposed model was derived with the use of more realistic assumptions in comparison with the literature known eq. (15). The model eq. (11) was tested in the experiments with the use of different analytes, columns and stationary

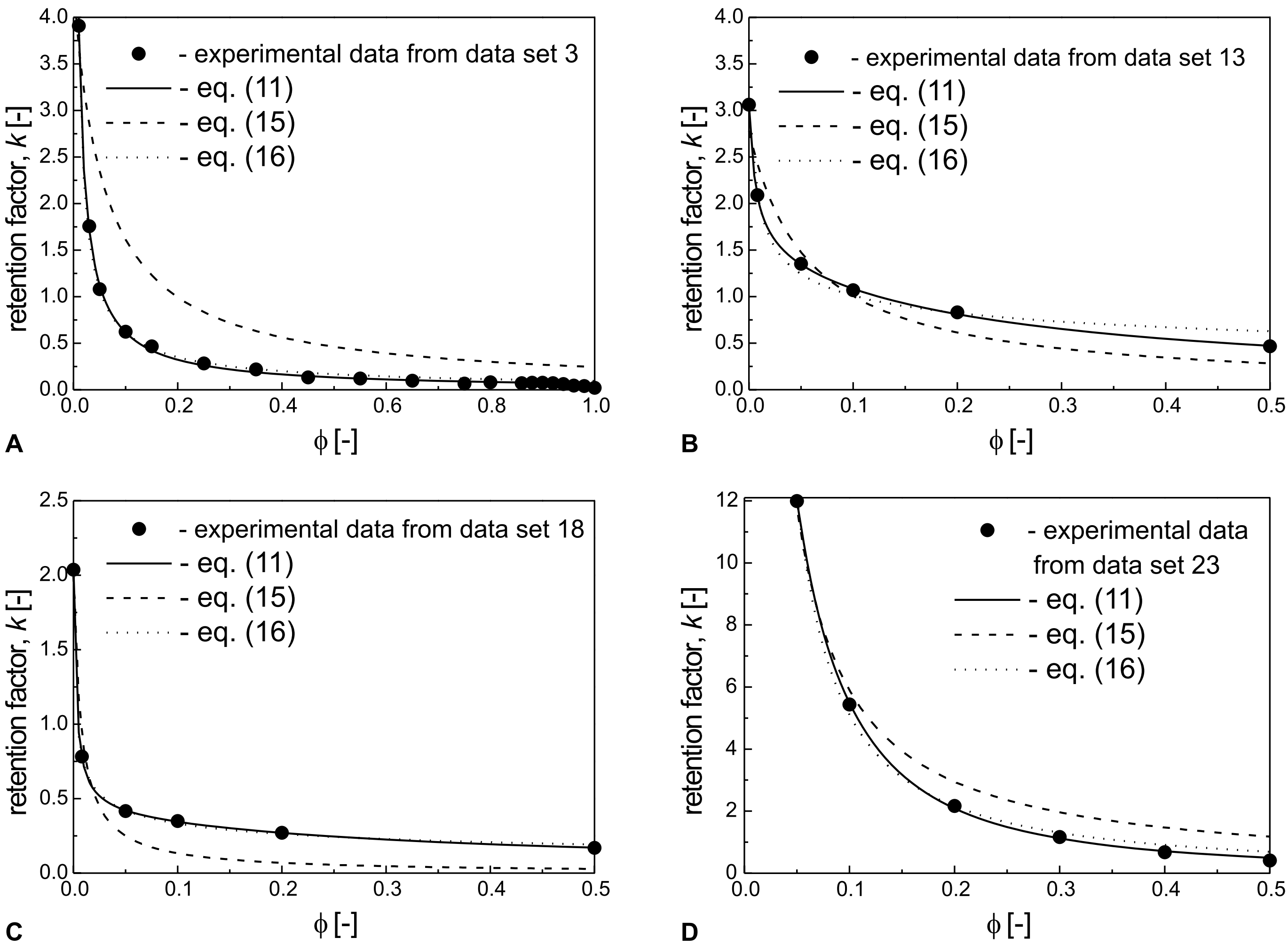

Figure 3. The example comparison of the experimental retention values (k) of the solutes with the theoretical data. The solid curves have been calculated from the proposed model, eq. (11). The dashed and dotted curves have been calculated from eq. (15) and eq. (16), respectively. A-data set 3, B-data set 13, C-data set 18, D-data set 23 (see Table 1).

phases including chemically modified and pure silica adsorbents.

– The obtained computation results confirm a very fine performance of the proposed model eq. (11). This model provides good fitting results and accuracy for most NP-HPLC systems tested in this work.

– Eq. (11) was compared with the two literature-known retention models developed by Kaczmarski et. all. and Snyder – Soczewiński. On the basis of the comparison of the statistical criteria for all tested retention models, it can be concluded that the five – parameters adsorption model (eq. (11)) gives significantly better fitting results than the other models (eq. (15 – 16)).

– The precise fitting results suggest that the proposed equation will be very usable in practical prediction and optimization of the mobile phase composition – it seems that this model permits to choose the optimal eluent composition from the data of few isocratic experiments.

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