



**SEMI-EMPIRICAL EQUATION FOR THE PREDICTION
OF RETENTION IN NORMAL PHASE LIQUID
CHROMATOGRAPHY (NP-LC) WITH POLAR BONDED
STATIONARY PHASES**

Wojciech ZAPAŁA^{1*)}, Lidia ZAPAŁA²⁾

¹⁾Department of Chemical and Process Engineering,

²⁾Department of Inorganic and Analytical Chemistry,
Chemical Faculty, Rzeszów University of Technology
Al. Powstańców Warszawy 6, 35-959 Rzeszów – Poland
e-mail: ichwz@prz.edu.pl

ABSTRACT

A semi-empirical heterogeneous adsorption model for the accurate prediction of analyte retention in a column chromatography with binary mobile phase on polar bonded adsorbent surface was proposed. The performance of the proposed equation was compared with two retention models reported in the literature. All models were verified for different LC systems by means of four criteria: the sum of squared differences between the experimental and theoretical data, approximation of standard deviation, Fisher test and F-test ratio.

Keywords: retention prediction, retention models, polar bonded stationary phase, NP-HPLC

INTRODUCTION

Chromatographic operations are usually carried out in isocratic mode, i.e., with constant temperature, pressure or solvent composition of the mobile phase during the process. However, it is well known that the adsorption behaviors of the system can strongly be affected by changes of these operating parameters. In liquid chromatography, the idea of affecting the adsorption behavior by modulation of the mobile phase composition is often exploited to improve separation performance, i.e., optimization, in chromatographic processes. Different methods to optimize chromatographic

* Corresponding author

separation, e.g., interpretative strategy [1], require the use of equations relating retention parameters to mobile phase composition (modifier concentration). The various retention models include theoretical (e.g., Snyder [2,3] and Soczewiński [4], Scott-Kucera [5], Jaroniec et al. [6,7]) as well as semi-empirical (e.g., Soczewiński and Wachtmeister [8], Shoenmakers et al. [9]) or empirical equations (e.g. Frost et al. [10], Zapała et al. [11]). These equations are widely used in computer-assisted optimization studies where their availability reduces the number of experimental observations required to determine the mobile phase composition that yields an optimum separation. It should be noted that from the practical prediction and optimization of the mobile phase composition it is not very important whether the retention model is rigorously theoretical, semi- or fully empirical – more important is the goodness of the fit of experimental data at changing mobile phase.

Recently, polar bonded phases have been increasingly used as adsorbents in liquid chromatography. Surface-modified sorbents (especially polar stationary phases bonded to silica matrix) having moderate polarities, they can be used both in normal and reversed-phase chromatography, bringing about the possibility of various separation selectivities. Three polar bonded types, diol (dihydroxypropyl ether), aminopropyl and cyanopropyl, are widely applied mainly in NP-LC [12-20]. These bonded phases exhibit similar properties to those of solid adsorbents, but they possess some important advantages over solid adsorbents, which include: very weak irreversible adsorption of strongly polar solutes, faster column equilibration, lesser importance of water content in the mobile phase and enhanced chromatographic selectivity.

In NP-LC systems with polar-bonded stationary phases it is assumed that the mechanism of solute retention strongly resembles that in adsorption liquid chromatography, with major role played by interactions between the intermolecular solute and stationary phase, involving terminal groups of chemically bonded ligands and the silanol groups of the non-bonded silica matrix. The most frequently employed chemically bonded stationary phases consist of the silica matrix with a certain amount of the surface silanols substituted by the organic ligands. For example, the density of coverage of the silica surface with 3-cyanopropyl ligands, which acts as a polar active centre, is equal (LiChrospher 100 CN 5 μm stationary phase produced by Merck, Darmstadt – Germany) to 3.52 [$\mu\text{mol}/\text{m}^2$] which means that only 41.9% of all silica silanols are substituted [21]. Thus, these $-\text{CN}$ and $\equiv\text{Si}-\text{OH}$ groups act as two different active sites on the stationary phase surface, able to participate on energetically different, but significant interactions with the polar moieties of solute molecules. From this point of view, it was justifiably assumed that the heterogeneity of the adsorbent surface may be important in

predicting retention in liquid chromatography, especially in systems with polar bonded stationary phases. For this reason, proposed in this paper is a semi-empirical two-site adsorption model, valid basically for the normal-phase liquid chromatography systems with chemically bonded stationary phases. The presented model was examined by means of different, chromatographic systems involving chemically bonded stationary phases. Moreover, the aim of this work was to analyse the accuracy and applicability of the proposed model in comparison with two most popular literature known retention models. Even though there are many models available, investigations to find more precise models are still being carried out, which was also the aim of this work.

THE SEMI-EMPIRICAL TWO-SITE ADSORPTION MODEL OF NP-HPLC

Let us consider the model of an ideal chromatographic column [22]:

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

where:

- c_i, Γ_i – are the concentrations of i -th component in 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,
- w – is the linear eluent flow velocity.

Assuming that the adsorption/desorption process is infinitely fast and the concentration of analyte, c_1 , is very low in comparison with the modifier and main component of eluent concentrations (c_2 and c_3 respectively), then changes of the concentration c_1 lead to practically negligible perturbations of the eluent component concentrations c_2 and c_3 . Thus, the time derivative of the surface analyte concentration can be approximated as follows:

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

Therefore, Equation (1), with reference to the analyte only, can be rewritten as follows:

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

where:

c_l, Γ_l – are the concentrations of the analyte in 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 linear velocity, w , the following can be written:

$$t_r = \frac{H}{w} \cdot \left(I + \frac{I - \varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma_l}{\partial c_l} \right) \quad (4)$$

or, after transforming Equation (4), the retention factor of analyte, k , can be described as follows:

$$k = \frac{t_r - t_0}{t_0} = \frac{I - \varepsilon_t}{\varepsilon_t} \cdot \frac{\partial \Gamma_l}{\partial c_l} \quad \text{where: } t_0 = \frac{H}{w} \quad (5)$$

For a two-site heterogeneous surface, the multicomponent adsorption can be described by the IAS (ideal adsorbed solution) model for each type of adsorption site separately and then the total adsorbed amount is a sum of the amounts adsorbed on each site [23]. Therefore, for chemically modified stationary phases, it was justifiably assumed, that the isotherm equation describing the sorption process of the constituents of the liquid phase can be defined as the sum of interactions of these components with the chemically bonded organic ligands and a sorption of the same components on the free active sites of the silica matrix. In multicomponent chromatographic systems, when an adsorbent surface is covered with two different kinds of sites we can account for the competitive behavior of three components using a bi-Langmuir competitive isotherm:

$$\Gamma_l = \frac{K_{I1} \Gamma_I^\infty c_1}{I + K_{I1} c_1 + K_{I2} c_2 + K_{I3} c_3} + \frac{K_{II1} \Gamma_{II}^\infty c_1}{I + K_{II1} c_1 + K_{II2} c_2 + K_{II3} c_3} \quad (6)$$

where:

c_1, c_2, c_3 – are the concentrations of the analyte and mobile phase compounds,

$\Gamma_I^\infty, \Gamma_{II}^\infty$ – are the saturation capacities of the two-site solid phase,

K_{Ii}, K_{IIi} – are the equilibrium constants of the chromatographic system compounds.

It should be noted, that the multicomponent Langmuir-type isotherms are thermodynamically consistent only if the saturation capacities are equal for all components of the chromatographic system. However, such isotherm models, treated as semi-empirical or empirical equations, are often applied by many authors (for example, see [24] and references therein) regardless of their thermodynamical inconsistency. These isotherms are mathematically very simple, they correctly describe a number of systems and are very easy to use in different mathematical models (e.g., sorption dynamics models). Thus, despite many different, more or less mathematically complicated isotherm models available in literature, in this work the competitive bi-Langmuir isotherm Equation (6) is applied in Equation (5) for derivation of the two-site retention model.

For low analyte concentrations (analytical mode) c_1 , Equation (6) can be written as follows:

$$\Gamma_1 = \frac{K_{I1}\Gamma_1^\infty c_1}{1 + K_{I2}c_2 + K_{I3}c_3} + \frac{K_{II1}\Gamma_{II}^\infty c_1}{1 + K_{II2}c_2 + K_{II3}c_3} \quad (7)$$

The derivative of Γ_1 on c_1 gives the following relation:

$$\frac{\partial \Gamma_1}{\partial c_1} = \frac{K_{I1}\Gamma_1^\infty}{1 + K_{I2}c_2 + K_{I3}c_3} + \frac{K_{II1}\Gamma_{II}^\infty}{1 + K_{II2}c_2 + K_{II3}c_3} \quad (8)$$

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

$$k = \frac{1 - \varepsilon_i}{\varepsilon_i} \cdot \left(\frac{K_{I1}\Gamma_1^\infty}{1 + K_{I2}c_2 + K_{I3}c_3} + \frac{K_{II1}\Gamma_{II}^\infty}{1 + K_{II2}c_2 + K_{II3}c_3} \right) \quad (9)$$

After converting the mobile phase compounds' molar concentrations into molar fractions, Equation (9) can further be rendered in the following form:

$$k = \frac{p'_1}{1 + p'_2\varphi + p'_3(1-\varphi)} + \frac{p'_4}{1 + p'_5\varphi + p'_6(1-\varphi)} \quad (10)$$

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

$$k = \frac{1 + p_1 \cdot \varphi}{p_2 + p_3 \cdot \varphi + p_4 \cdot \varphi^2} \quad (11)$$

where:

p_1, p_2, p_3, p_4 – are the experimental equation parameters. Note that only positive values of the parameters should be taken into consideration.

Because the two-site isotherm model used in this work and given by Equation (6) is thermodynamically inconsistent, the proposed Equation (11) should be treated as a semi-empirical model. The aim of this work was to verify the accuracy of the presented model Equation (11) for the description of retention processes in different NP-HPLC systems with polar bonded stationary phases. For experimental verification of the proposed model Equation (11), the literature data [25-27] from NP-HPLC measurements were employed. Table 1 specifies the example samples, mobile phases, the range of modifier volume or mole fractions, and the type of chromatographic columns used (three different types of polar bonded adsorbents were tested: cyanopropyl, diol (dihydroxypropyl ether) and aminopropyl). The test solutes presented in Table 1 represent a wide variety of chemical structures with different capacities able to interact intermolecularly with the active sites of stationary phase. The detail conditions used for measuring the experimental data are available in papers [25-27].

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 [28]. The accuracy of determination of model parameters was assessed for the 95% confidence interval of the Student's test. The following statistical criteria were used for the assessment of proposed model accuracy in different HPLC systems:

- 1) sum of squared differences between the experimental and theoretical retention data:

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

- 2) approximation of standard deviation:

$$SD = \sqrt{\frac{SUM}{N-l}} \quad (13)$$

- 3) Fisher test:

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

where:

- $i = 1 \dots N$, N – number of experimental points,
- l – number of estimated model parameters.

From the values of the presented statistical criteria the goodness of fit of experimental data to the particular model equation was verified.

Table 1. Datasets examined in the present study.

Set	Test analyte	Mobile phase	Range of modifier volume or mole fraction	Column
1	4,4'(5')-di-tert-butyl-dibenzo[14]-crown-4	2-propanol – n-hexane	0.01 – 0.8 (2-propanol)	LiChrospher 100 CN 125 x 4 mm, 5 µm Merck, Darmstadt Germany
2	1-naphthol		0.01 – 1.0 (2-propanol)	
3	p-cresol		0.01 – 0.8 (2-propanol)	
4	2,3-dimethylphenol		0.01 – 0.8 (2-propanol)	
5	1,3,5-trihydroxybenzene		0.1 – 0.8 (2-propanol)	
6	1-naphthylamine		0.05 – 0.8 (2-propanol)	
7	quinoline		0.05 – 0.8 (2-propanol)	
8	ethyl acetate	pentane – diethyl ether	0.008 – 0.5 (diethyl ether)	Hypersil Cyanopropyl 250 x 4.6 mm, 5 µm Shandon, Eragny France
9	butyl isothiocyanate			
10	phenol			
11	γ-decalactone			
12	ethyl acetate	pentane – diethyl ether	0.008 – 0.5 (diethyl ether)	LiChrospher 100 Diol 250 x 4.6 mm, 5 µm Shandon, Eragny France
13	ethyl(E)-2-butenoate			
14	butyl isothiocyanate			
15	γ-decalactone			
16	2-undecanone			
17	triphenylene			
18	ethyl acetate	pentane – diethyl ether	0.008 – 0.5 (diethyl ether)	Hypersil Aminopropyl 250 x 4.6 mm, 5 µm Shandon, Eragny France
19	ethyl(E)-2-butenoate			
20	butyl isothiocyanate			
21	triphenylene			
22	phenanthrene			

RESULTS AND DISCUSSION

Table 2 specifies the values of estimated model parameters p_i and the Fisher test values obtained as a result of comparison between the proposed model (Equation 11) and experimental data. The related sums of squared differences between the experimental and theoretical data, and SDs are presented in Figs. (1-2). Figs. (3-6) also include typical graphical comparisons of experimental retention values (k) of solutes with theoretical data.

On the basis of comparison between the theoretical and experimental data presented in Table 2 and in Figs. (1-6), it can be concluded that the four-parameter model proposed in this study (Equation 11) provides excellent agreement between the experimental and theoretical data for most of the NP chromatographic systems studied (especially for systems with strong nonlinear $k = f(\varphi)$ dependence – see example Fig. 3). The related values of SUMs and SDs are very low (see Figs. 1-2) and the Fisher test values are in many cases larger than 10^3 (see Table 2).

The second purpose of this work was a comparison of the proposed heterogeneous model (Equation (11)) with two retention models known in literature and most widely used in NP-HPLC in systems with pure silica or alumina adsorbents:

- the retention model derived from the Snyder-Soczewiński theory assumes monolayer adsorption of a polar component of the eluent on the adsorbent surface and their displacement by molecules of the chromatographed compounds [2-4]:

$$\ln k = p_1 - p_2 \cdot \ln \varphi \quad (15)$$

- the model proposed by Scott and Kucera assumes bilayer adsorption of solvent, sorption of solute molecules without displacement as well as dispersive interactions between eluent components and solute molecules [5]:

$$k = \frac{I}{p_1 + p_2 \cdot \varphi} \quad (16)$$

All models were compared in different NP-HPLC systems presented in Table 1, by means of three statistical criteria (Equations (12)-(14)). In Figs. 1-2, the values of the sums of squared differences between the experimental and theoretical data, and SDs for all models tested in this work were compared. Table 2 specifies also the values of estimated model parameters and the Fisher test values obtained as a result of comparison between Equations (15-16) and the experimental data. The dashed lines in Figures 3-6 show the theoretical curves obtained from Equations (15-16). From comparison of the three statistical parameters (see Figs. 1-2 and Table 2), one can see that Equation (11) much better describes the experimental data tested in this work, giving lower values of SUMs and SDs and higher values of Fisher tests, compared with Equations (15-16) proposed in [2-5].

Table 2. Estimated numerical values of Equations (11) and (15-16) and F-test values.

Set	Model M ₁ Equation (11)				Model M ₂ Equation (15)				Model M ₃ Equation (16)				
	p_1	p_2	p_3	p_4	p_1	p_2	p_3	p_4	p_1	p_2	p_3	p_4	F
1	6.508±1.7	0.1818±0.02	8.742±2.6	7.694±4.04	428.9	-0.674±0.1	0.4506±0.02	158.4	0.2327±0.04	3.859±1.02	23.13		
2	8.349±2.4	0.0±0.0	14.21±0.3	75.62±12.0	3435.0	-2.36±0.07	0.9433±0.02	1960.0	0.01312±8E-3	12.51±0.8	1048.0		
3	63.98±15.5	0.0546±1e-4	27.4±9.6	906.6±142.7	3.0e4	-0.914±0.4	0.3325±0.04	46.59	0.05457±6E-4	19.19±1.1	2677		
4	45.05±5.0	0.1141±5e-4	31.11±7.0	678.3±76.1	1.8e4	-2.362±0.1	0.7509±0.4	193.2	0.1143±0.02	21.08±1.4	969.6		
5	0.1967±0.01	0.0±0.0	0.6949±0.02	11.47±1.4	1.3e4	-2.19±0.07	1.696±0.03	4640.0	-0.2583±0.1	4.371±1.0	86.12		
6	0.0±0.0	0.2225±0.004	3.942±0.28	0.6108±0.3	768.5	-1.27±0.1	0.7234±0.05	51.87	0.2222±4E-3	4.055±0.15	547.2		
7	1.0±0.00001	0.2777±0.04	4.37±0.7	8.187±2.0	310.0	-1.513±0.1	0.7565±0.06	50.48	0.2254±0.03	5.213±0.3	184.1		
8	27.21±0.001	2.206±0.001	161.6±0.001	200.7±0.001	1.4e6	-2.335±0.1	0.2654±0.04	279.7	2.924±1.5	19.18±3.0	7.5		
9	11.37±0.001	2.818±0.01	49.33±0.001	112.5±0.001	9.1e6	-2.019±0.8	0.2031±0.2	6.8	2.897±0.2	11.54±3.0	232.0		
10	0.0±0.0	0.05956±0.005	11.03±0.7	1.312±0.5	3.4e5	-1.957±0.5	0.8015±0.1	1267.0	0.0589±0.006	11.13±0.7	2.48e4		
11	292.2±73.1	0.0±0.0	63.04±10.0	578.1±18.2	7760.0	-0.047±0.9	0.03897±0.2	34.93	0.1443±0.04	2.532±1.9	50.06		
12	4.83±0.001	0.9929±0.0001	28.19±0.001	29.9±0.001	3.4e6	-1.904±0.8	0.3647±0.2	35.99	1.064±0.3	16.02±10.0	57.95		
13	2.23±0.3	0.8637±0.01	25.24±1.6	0.0±0.0	1.4e4	-1.948±0.8	0.3696±0.17	50.1	0.9179±0.3	17.53±12.2	58.9		
14	21.37±0.001	1.907±0.001	104.0±0.001	93.19±0.0001	5.3e6	-2.011±0.1	0.2413±0.04	305.8	2.418±1.2	12.56±11.0	6.66		
15	252.5±22.4	0.0±0.0	33.86±0.0001	596.6±64.1	2671.0	-0.274±0.2	0.5302±0.1	116.3	0.07902±0.0005	2.932±0.5	682.3		
16	1.858±0.1	0.7393±0.005	25.97±0.6	0.0±0.0	1.01e5	-2.059±0.8	0.4431±0.2	66.89	0.7802±0.2	19.85±11.1	103.6		
17	62.26±20.0	0.3445±0.1	45.12±1.3	180.5±10.1	1858.0	-0.670±0.4	0.2961±0.1	25.63	0.4576±0.09	4.43±2.0	31.06		
18	8.392±0.001	0.8236±0.0	72.42±0.001	59.71±0.0001	3.2e7	-2.66±0.08	0.4938±0.02	9690.0	1.021±0.5	38.32±30.0	36.78		
19	9.418±0.001	0.6964±0.0	77.76±0.001	70.04±0.0001	9.7e6	-2.723±0.1	0.521±0.03	4489.0	0.9131±0.5	40.46±40.1	39.1		
20	7.23±0.001	2.603±0.001	64.56±0.001	45.08±0.0001	3.1e6	-2.371±0.5	0.2707±0.1	39.33	2.91±1.1	20.68±9.8	16.12		
21	108.5±12.0	0.0±0.0	119.3±6.0	484.6±9.7	5507.0	-1.326±0.2	0.4076±0.05	232.8	0.4583±0.1	9.991±6.3	20.1		
22	138.2±6.4	0.0±0.0	328.1±8.2	993.0±81.0	1.9e4	-1.884±0.2	0.3402±0.04	183.0	1.192±0.4	16.57±12.2	12.24		

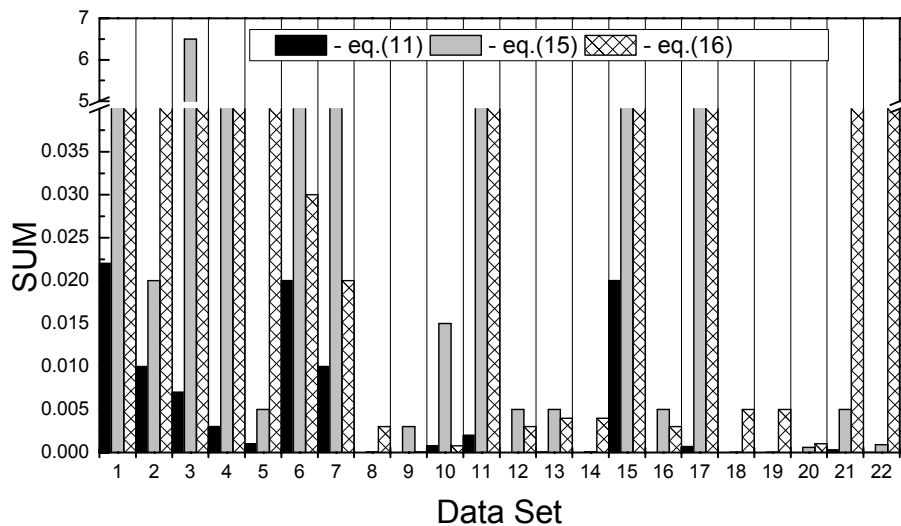


Fig. 1. Graphical comparison of sums of squared differences between experimental and theoretical retention data.

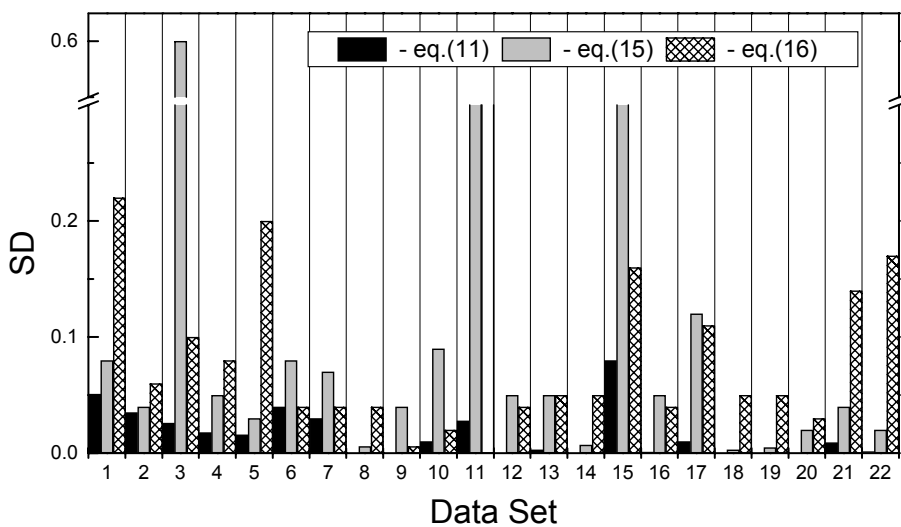


Fig. 2. Graphical comparison of numerical values of standard deviation for studied retention models and data sets presented in Table 1.

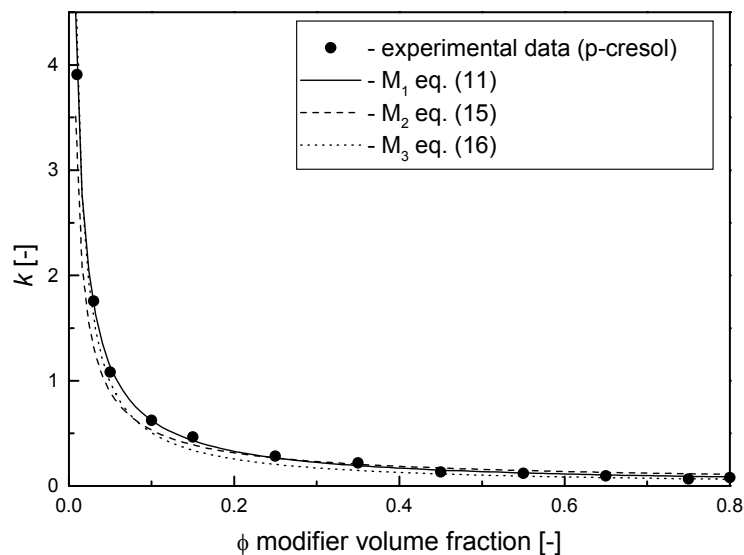


Fig. 3. Comparison of retention values (k) of solutes of data set 3 with theoretical data. Solid curves have been calculated from proposed model, Equation (11). Dashed and dotted curves have been calculated from Equation (15) and Equation (16), respectively.

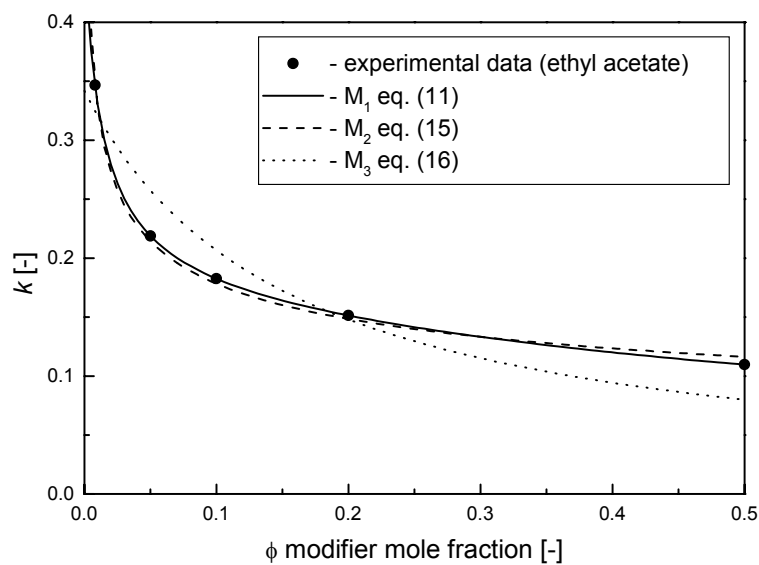


Fig. 4. Comparison of retention values (k) of solutes of data set 8 with theoretical data. Solid curves have been calculated from proposed model, Equation (11). Dashed and dotted curves have been calculated from Equation (15) and Equation (16), respectively.

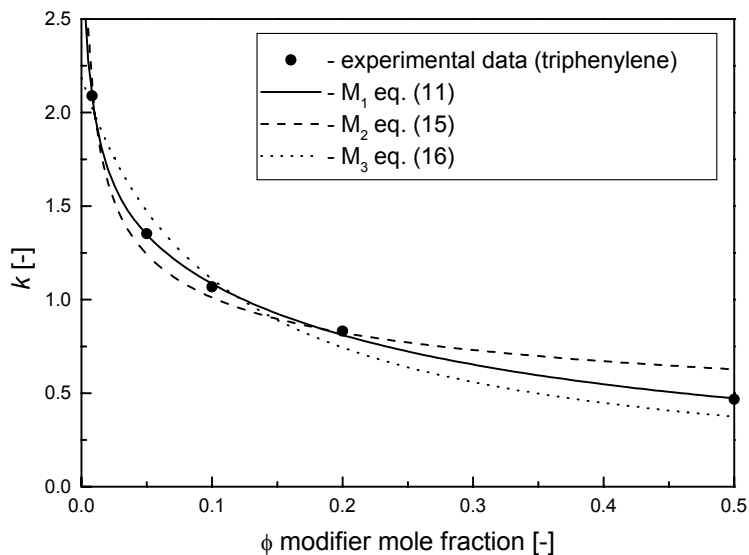


Fig. 5. Comparison of retention values (k) of solutes of data set 17 with theoretical data. Solid curves have been calculated from proposed model, Equation (11). Dashed and dotted curves have been calculated from Equation (15) and Equation (16), respectively.

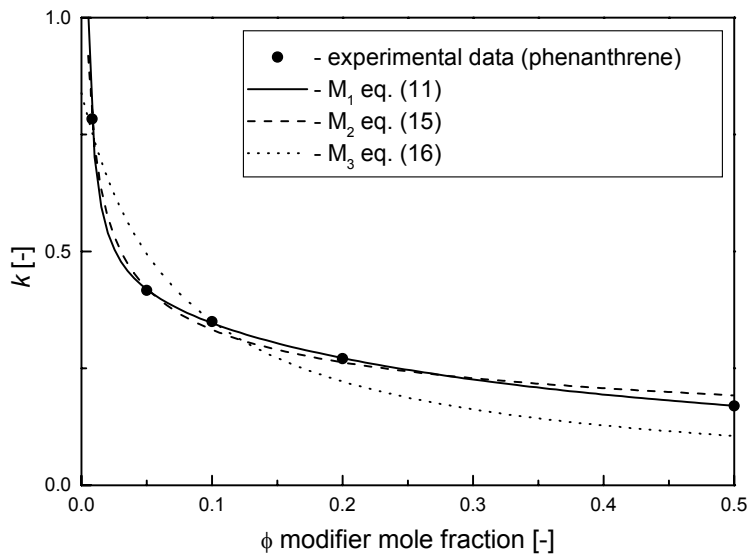


Fig. 6. Comparison of retention values (k) of solutes of data set 22 with theoretical data. Solid curves have been calculated from proposed model, Equation (11). Dashed and dotted curves have been calculated from Equation (15) and Equation (16), respectively.

Although, on the basis of the statistical criteria used, the accuracy of the models tested in this work can be verified. Moreover, in order to compare models that have different numbers of parameters and to determine which model is evidently better, more sophisticated statistical tools, such as the *F-test* ratio, should be used. Between the two models M_1 and M_2 , the *F-test* ratio, F_{M_1, M_2} , is calculated by:

$$F_{M_1, M_2} = \frac{F_{M_1}}{F_{M_2}} \quad (17)$$

where:

F_{M_1}, F_{M_2} – are the Fisher test values for models M_1 and M_2 , respectively.

Taking into account a risk α , the model M_1 correlates better the experimental data than the model M_2 if:

$$F_{M_1, M_2} \geq F_{N-l_1, N-l_2, \alpha} \quad (18)$$

where:

- N – number of experimental points,
- l_1, l_2 – are the numbers of adjusted parameters in the models M_1 and M_2 , respectively,
- $F_{N-l_1, N-l_2, \alpha}$ – is available in statistical test tables.

In Table 3 the, F_{M_1, M_i} , *F-test* ratio values are presented, where M_1 relates to Equation (11) and M_i ($i = 2, 3$) relate to Equations (15-16), respectively. Table 3 specifies also the $F_{N-l_1, N-l_2, 1\%}$ values taken from statistical test tables. If Equation (18) is true at a risk α of 1% (see Table 3), then the model M_1 is statistically evidently better than the other models $M_2 - M_3$. As it can be seen in Table 3:

- only for 1, 2, 5, 15 and 21 (5 cases out of 22) data sets, the condition given by Equation (18) is not fulfilled in the case of comparisons of Equation (11) and Equation (15),
- only for 6, 7, 10 and 15 (4 cases out of 22) data sets, the condition given by Equation (18) is not fulfilled in the case of comparisons of Equation (11) and Equation (16).

For other systems tested (see Table 3), the results show that the proposed two-site model Equation (11) is statistically better than models (15-16).

Recapitulating, in the case of NP-HPLC systems with polar bonded adsorbents, taking into account all statistical criteria, the proposed heterogeneous model (Equation (11)) very well describes quantitatively the process studied and is evidently better than other theoretical models tested in this work.

Table 3. Comparisons of proposed model Equation (11) with Equations (15-16) with the aid of *F-test* ratio.

Set	N	$F_{N-4, N-2, 1\%}$	Eq. (11) vs. Eq. (15)		Eq. (11) vs. Eq. (16)	
			F_{M_1, M_2}	Eq. (18)	F_{M_1, M_3}	Eq. (18)
1	12	5.06	2.71	false	18.54	true
2	20	3.19	1.75	false	3.28	true
3	13	4.6	643.92	true	11.21	true
4	13	4.6	93.17	true	18.56	true
5	9	7.46	2.80	false	150.95	true
6	17	3.6	14.82	true	1.4	false
7	16	3.8	6.14	true	1.68	false
8	5	34.12	5005.4	true	1.86e6	true
9	5	34.12	1.34e6	true	3.92e4	true
10	5	34.12	268.35	true	13.71	false
11	5	34.12	222.16	true	155.01	true
12	5	34.12	9.45e4	true	5.87e4	true
13	5	34.12	279.44	true	237.69	true
14	5	34.12	1.73e4	true	7.96e5	true
15	5	34.12	22.97	false	3.91	false
16	5	34.12	1.51e3	true	974.90	true
17	5	34.12	72.49	true	59.82	true
18	5	34.12	3.30e3	true	8.70e5	true
19	5	34.12	2.16e3	true	2.48e5	true
20	5	34.12	7.88e4	true	1.92e5	true
21	5	34.12	23.66	false	273.98	true
22	5	34.12	103.82	true	1.6e3	true

CONCLUSION

Quantitative retention versus eluent composition relationships are of fundamental importance for method development in chromatography. Therefore, in this study a semi-empirical equation was proposed for description of the retention coefficient, k , of a given solute as a function of the mixed mobile phase composition. The model Equation (11) was tested in the experiments with the use of different analytes, columns and sorbents with chemically bonded ligands. The computation results obtained confirm the very fine performance of the proposed model Equation (11). This model provides good fitting results and accuracy for most NP-HPLC systems tested in this work.

The model Equation (11) was compared with two literature-known retention models developed by Snyder – Soczewiński and Scott – Kucera. On the basis of comparison of the statistical criteria for all of the retention models tested, it can be concluded that the two-site adsorption model (Equation (11)) gives much better fitting results than the other models (Equation (15-16)). Besides, the precisely fitting results suggest that the proposed equation will be very useful in the practical prediction and optimization of the mobile phase composition – it seems that this semi-empirical model permits to choose the optimal eluent composition from the data of several isocratic experiments.

Although the proposed model (Equation (11)) is a semi-empirical equation, the results of this study show that, especially in normal phase chromatographic systems with chemically modified stationary phases, the heterogeneity of the adsorbent surface should be taken into consideration in analysis of the influence of modifier concentration on the analyte retention process.

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