Influence of Mobile Phase Composition on Retention Factors in Different HPLC Systems with Chemically Bonded Stationary Phases

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Abstract

The influence of mobile phase composition on the retention of selected test analytes in different normal- and reversed-phase chromatographic systems is studied. A novel adsorption model for an accurate prediction of the analyte retention in the column chromatography with binary mobile phase is proposed. Performance of the model is compared with the retention model reported in the literature. Both models are verified for different HPLC systems by use of three criteria: (*a*) the sum of squared differences between the experimental and theoretical data, (*b*) approximation of the standard deviation, and (*c*) the Fisher test.

Introduction

The study of the influence of a mobile phase modifier concentration on the retention in high-performance liquid chromatography (HPLC) is important for understanding the regularities of the retention and mechanisms of substances separation in a chromatographic process. Binary eluents consisting of a weak and a strong solvent are widely used in HPLC. In the reversed-phase (RP) liquid chromatography (LC) mode, for example, the major constituent of the mixed eluent is a highly polar solvent (e.g., water), whereas a less polar solvent (e.g., methanol, acetonitrile, etc.) is an organic modifier added to control the process of elution. The composition of the mobile phase determines the retention volume and time of solutes, both in the normal-phase (NP) and RP chromatographic modes. Composition changes and the nature of mobile phases enable tuning of the separated analytes' retention in a wide range of the retention parameters and optimization of the chromatographic processes, as well. One factor, which undoubtedly contributes to the quality of separation, is selectivity. Among the factors that strongly affect the selectivity of separation in LC is the composition of the mobile phase. Optimization of separation selectivity can be achieved by several different methods, one of them is the so-called interpretative

strategy (1,2). The key role in this strategy is the implementation of adequate models of retention that couple the retention of a solute with the composition of a mixed eluent (3,5,6,9,13). The comparison of literature-known retention models for different thin-layer chromatography (TLC) and HPLC systems was presented in detail in a previous work (6). The results of the investigations showed that for a strong parabolic $k = f(\varphi)$ dependence (for crown compounds), a majority of retention models [except models proposed by Schoenmakers (1) and Kaczmarski et al. (2,5)] gave significant errors of computation. At the same time, the models correctly describing strong nonlinear $k = f(\varphi)$ dependences gave meaningful errors for low concentration of organic modifier. Therefore, the search for the most universal and accurate retention models for describing both linear and strong parabolic $k = f(\varphi)$ dependences is reasonable, from the practical and theoretical point of view.

In this paper, a novel adsorption model for an accurate prediction of the analyte retention in the column chromatography with binary mobile phase is proposed. This model has been formulated theoretically, according to methodology reported in literature (5). The presented model was experimentally examined by the author. In addition, the retention data taken from literature [and contained in papers by Kaczmarski et al. (5), Kahie et al. (7), Lanin et al. (8), and Nikitas et al. (9)] have been also used for the examination.

Theory

In the case of chemically bonded stationary phases, some of the surface silanols remain nonbonded. For such stationary phases, the isotherm equation $\Gamma(c)$, describing adsorption of the constituents of the liquid phase, can be written as follows:

$$\Gamma_1 = \Gamma_1' + \Gamma_1'' \qquad \text{Eq. 1}$$

where Γ_1' describes the interaction of these components with the chemically bonded organic ligands, and Γ_1'' is a sorption of the same components on the free active sites of the silica matrix.

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Let us assume a linear dependence of the amount of solute adsorbed on the chemically bonded ligands and its concentration in the mobile phase:

$$\Gamma_1 = K \cdot c_1 \qquad \text{Eq. 2}$$

The equation constant K is a function of the mole or volume fraction φ of a component of the binary mobile phase for different mobile phase compositions.

Function Γ_1'' describes adsorption on the free active sites of the silica matrix. The relation between the amount of the substance adsorbed on the free active sites of the sorbent and its concentration in the bulk solution can be given by the competitive Langmuir isotherm:

$$\Gamma_1'' = \frac{K_1 \Gamma^\infty c_1}{1 + K_1 c_1 + K_2 c_2 + K_3 c_3} \qquad \text{Eq. 3}$$

where c_1, c_2, and c_3 are the concentrations of the solute and the components of the mixed mobile phase, respectively; Γ^∞ is the saturation capacity of the solid phase; and K_1, K_2, and K_3 are the equilibrium constants. For low numerical values of c_1, equation 3 can be given as follows:

$$\Gamma_1'' = \frac{K_1 \Gamma^\infty c_1}{1 + K_2 c_2 + K_3 c_3} \qquad \text{Eq. 4}$$

In the case of column chromatography, the retention factor k is defined as follows:

$$k = \frac{t_r - t_0}{t_0} \qquad \text{Eq. 5}$$

On the basis of the model of an ideal chromatographic column (10), the retention coefficient (k) equals the ratio of the derivative of the solute concentration in the bulk mobile phase and the derivative of the solute concentration in the surface mobile phase:

$$k = \frac{1 - \varepsilon_1}{\varepsilon_1} \cdot \frac{\partial \Gamma_1}{\partial c_1} \qquad \text{Eq. 6}$$

From equations 1, 2, and 4, one can deduce that the sum of the derivatives of Γ_1' and Γ_1'' on c_1 can be given by the following relationship:

$$\frac{\partial \Gamma_1}{\partial c_1} = K + \frac{K_1 \Gamma^\infty}{1 + K_2 c_2 + K_3 c_3} \qquad \text{Eq. 7}$$

Let us assume that the parameter K in the first segment of equation 7 depends on the mole or volume fraction φ of the mobile phase modifier, according to the Langmuir-type model proposed by Row et al. (3,4):

$$K = p''_1 + \frac{p''_2}{\varphi} \qquad \text{Eq. 8}$$

where p''_1 and p''_2 are the experimental coefficients. This equa-

tion assumes that the adsorption of organic modifier is described by the Langmuir isotherm. The intercept, p''_1, characterizes the adsorption interaction between the organic modifier molecules and adsorbent surface, whereas the slope, p''_2, relates to the solute molecules and adsorbent surface interaction.

Combining equations 6–8, we can obtain:

$$k = \frac{1 - \varepsilon_1}{\varepsilon_1} \cdot \left(p''_1 + \frac{p''_2}{\varphi c_1} + \frac{K_1 \Gamma^\infty}{1 + K_2 c_2 + K_3 c_3} \right) \qquad \text{Eq. 9}$$

Assuming that the mixed mobile phase can be considered as an ideal mixture, equation 9 can be given in the following form:

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

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:

$$k = \frac{p_1 \cdot \left(1 + p_2 \cdot \varphi + \frac{p_3}{\varphi} \right)}{1 + p_4 \cdot \varphi} \qquad \text{Eq. 11}$$

Model parameters (p_i) were estimated by minimization of a sum of the squared differences between the experimental and theoretical data using the Marquardt method, which was later modified by Fletcher (11). The accuracy of determination of the model's parameters was assessed using the formulas given in the monograph (12) for the 95% confidence interval of Student's test.

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

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

$$\text{SUM} = \sum \left(k_{\exp}(i) - k_{\text{theor}}(i) \right)^2 \qquad \text{Eq. 12}$$

Approximation of standard deviation (SD):

$$\text{SD} = \sqrt{\frac{\text{SUM}}{\text{LD} - \text{L}}} \qquad \text{Eq. 13}$$

Fisher test:

$$F = \frac{\sum\limits_i \frac{\left(k_{\exp}(i) - \sum \frac{k_{\exp}(i)}{\text{LD}} \right)^2}{\text{LD} - 1}}{\sum\limits_i \frac{\left(k_{\exp}(i) - k_{\text{theor}}(i) \right)^2}{\text{LD} - \text{L}}} \qquad \text{Eq. 14}$$

where: $i = 1...LD$, LD = number of experimental points and L = number of estimated parameters.

Experimental

Table I specifies the samples, mobile phases, range of the modifier mole (or volume) fractions (separately for each mobile phase), and the mode of HPLC employed (NP- or RP-HPLC columns).

Chrysin and quercetin were purchased from Sigma (St. Louis, MO). LiChrosolv chromatographic-grade methanol and water were purchased from Merck (Darmstadt, Germany).

HPLC was performed with a Merck–Hitachi model L-7100 LaChrom pump, Merck–Hitachi model L-7455 DAD LaChrom detector, Merck–Hitachi model D-7000 LaChrom interface, Merck–Hitachi model L-7350 column oven, Merck model L-7612 solvent degasser, a 20-µL injection loop, and a Hypersil BDS C_{18} chromatography column [250 mm × 4.6 mm, 5-µm average particle diameter (d_p)] (Thermo Hypersil-Keystone, Cheshire, UK). The mobile phase flow rate was 1 mL/min, the absorbance was measured at 250 nm, and the column temperature was 20ºC. Elution was performed in the isocratic mode.

Results and Discussion

The results of the author's investigations in different HPLC systems are presented in example Figures 1–5 and in Table II. Table II specifies the values of estimated model parameters p_i and related sums of the squared differences between experimental and theoretical data, SDs, and the Fisher test values obtained as a result of comparison between the newly proposed model (equation 11) and the experimental data. From the presented results of the investigations, it can be concluded that the four–parameter model proposed in this study (equation 11) provides an excellent agreement between the experimental and theoretical data for most NP and RP chromatographic systems studied. It performs equally well both at high and low levels (data sets: 6, 9–12; Figures 2A and 3) of the organic modifiers. For the RP-HPLC of crown compounds as test analytes (data sets 15–18 and Figure 5), the proposed adsorption model (equation 11) describes these data sets absolutely satisfactorily.

In the second stage of the author's investigations, the comparison of the newly proposed model (equation 11) with the literature-known adsorption model (equation 15) developed by Nikitas et al. (9,13) was conducted:

		Mobile phase	**Range of modifier**		
Set	**Test analyte**	**composition**	**mole fraction**	**Column**	**Reference**
1	Chrisin	Water–methanol	0.20–1.00 (methanol)	250 mm × 4.6 mm, 5-µm Hypersil BDS C_{18}	Present work
2	Quercetin	Water–methanol	0.30–1.00 (methanol)	250 mm × 4.6 mm, 5-µm Hypersil BDS C_{18}	Present work
3	Apigenin*	Water–methanol*	0.31–0.64 (methanol)	250 mm × 4.5 mm, 7-µm LiChrosorb C_8 (Merck)	(7)
4	Chryseriol*	Water–methanol*	0.31–0.64 (methanol)	300 mm × 3.9 mm, 10-µm Phenyl µBondapak (Waters)	(7)
5	Flavonol*	Water–methanol*	0.31–0.64 (methanol)	300 mm × 3.9 mm, 10-µm µBondapak C_{18} (Waters)	(7)
6	L-Tryptophan	Water–isopropoanol	0.004–0.2 (isopropanol)	250 mm × 4.0 mm, 5-µm Intersil ODS-3	(9)
7	Clarithromycin	Water–methanol	0.575–0.825 (methanol)	250 mm × 4.6 mm, 5-µm Kromasil C_{18}	(9)
8	Roxithromycin	Water–acetonitrile	0.3–0.6 (acetonitrile)	250 mm × 4.6 mm, 5-µm Kromasil C_{18}	(9)
9	Ethylbenzene	n-Hexane–THF	$8 \times 10^{-5} – 3.21 \times 10^{-3}$ (THF)	120 mm × 2 mm, 10-µm Silasorb-NH_2 (Milichrom)	(8)
10	Hexylbenzene	n-Hexane–THF	$8 \times 10^{-5} – 3.21 \times 10^{-3}$ (THF)	120 mm × 2 mm, 10-µm Silasorb-NH_2 (Milichrom)	(8)
11	o-Xylen	n-Hexane–THF	$8 \times 10^{-5} – 3.21 \times 10^{-3}$ (THF)	120 mm × 2 mm, 10-µm Silasorb-NH_2 (Milichrom)	(8)
12	1,2,4,5-Tetramethylbenzene	n-Hexane–THF	$8 \times 10^{-5} – 3.21 \times 10^{-3}$ (THF)	120 mm × 2 mm, 10-µm Silasorb-NH_2 (Milichrom)	(8)
13	1-Naphthol†	2-Propanol–n-hexane	0.5–1.0 (v/v, propanol)	119 mm × 4 mm, 5-µm LiChrospher 100 CN (Merck)	(5)
14	m-Cresol†	2-Propanol–n-hexane	0.5–1.0 (v/v, propanol)	119 mm × 4 mm, 5-µm LiChrospher 100 CN (Merck)	(5)
15	Dibenzo-24-crown-8†	Water–methanol	0.6–1.0 (v/v, methanol)	119 mm × 4 mm, 5-µm LiChrospher 100 RP-8 (Merck)	(5)
16	Dibenzo-24-crown-8†	Water–methanol	0.6–1.0 (v/v, methanol)	119 mm × 4 mm, 5-µm LiChrospher 100 RP-8e (Merck)	(5)
17	Dibenzo-24-crown-8†	Water–methanol	0.6–1.0 (v/v, methanol)	119 mm × 4 mm, 5-µm LiChrospher 100 RP-18 (Merck)	(5)
18	Dibenzo-24-crown-8†	Water–methanol	0.6–1.0 (v/v, methanol)	119 mm × 4 mm, 5-µm LiChrospher 100 RP-18e (Merck)	(5)

* Acetic acid as acidic modifier.
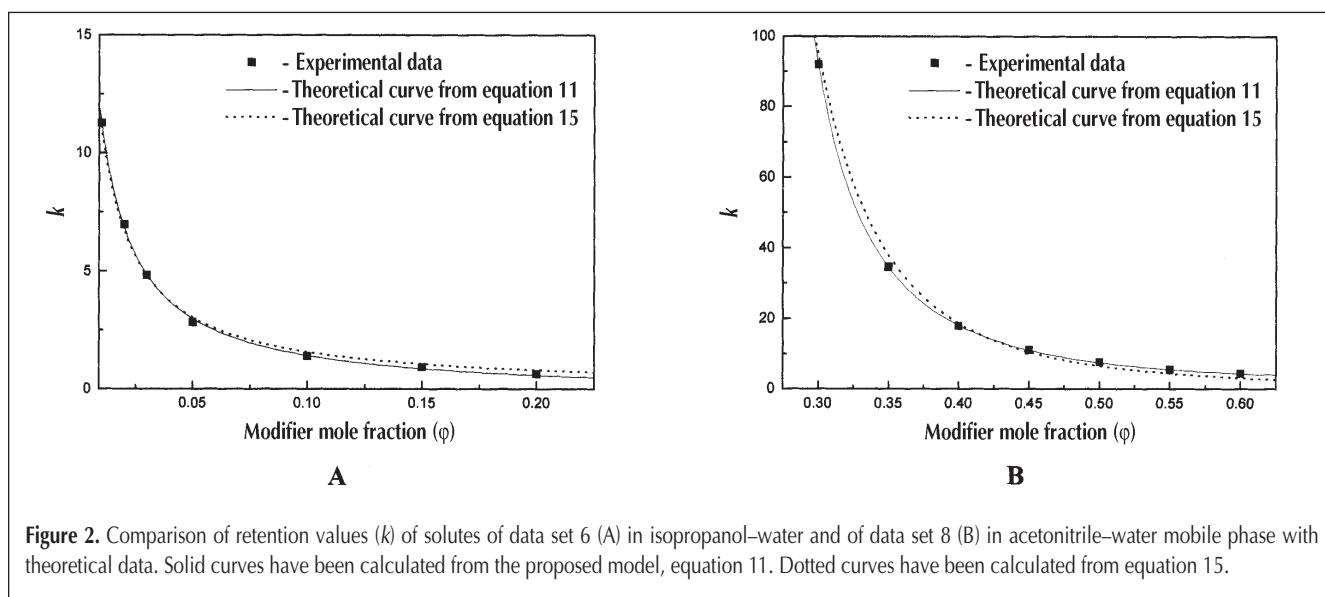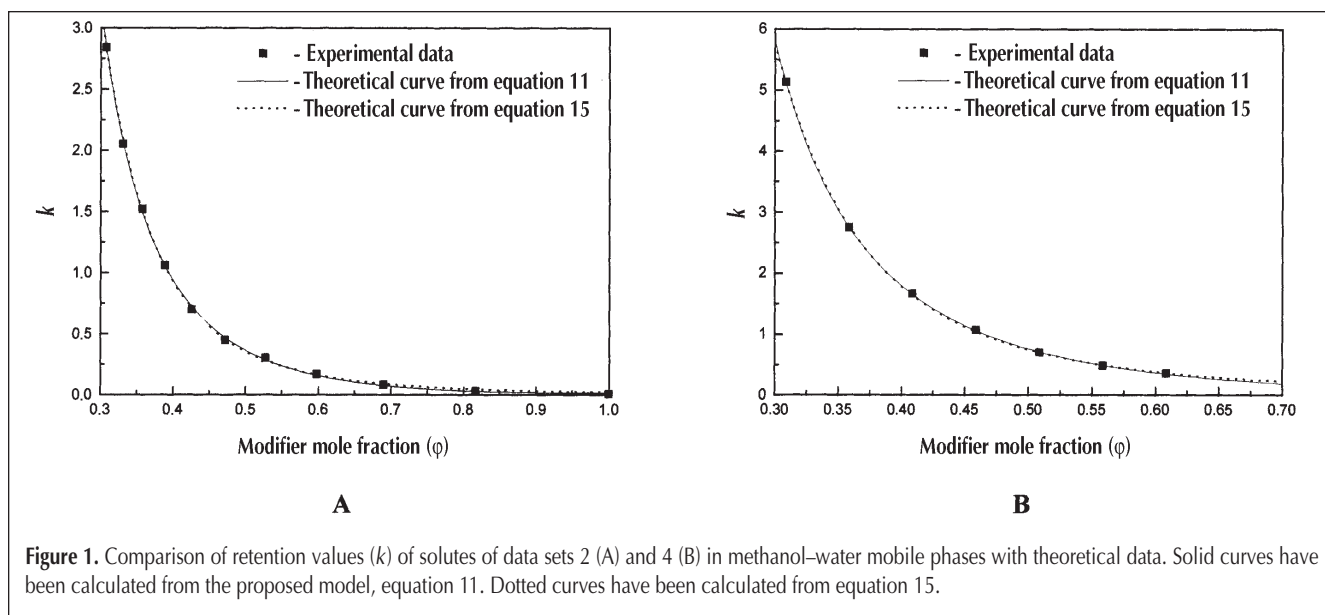† Volume proportions, rather than mole fractions, are given.

$$k = exp\left[p_1 - ln\,(1 + p_2 \cdot \varphi) - \frac{p_3 \cdot \varphi}{1 + p_2 \cdot \varphi}\right]$$
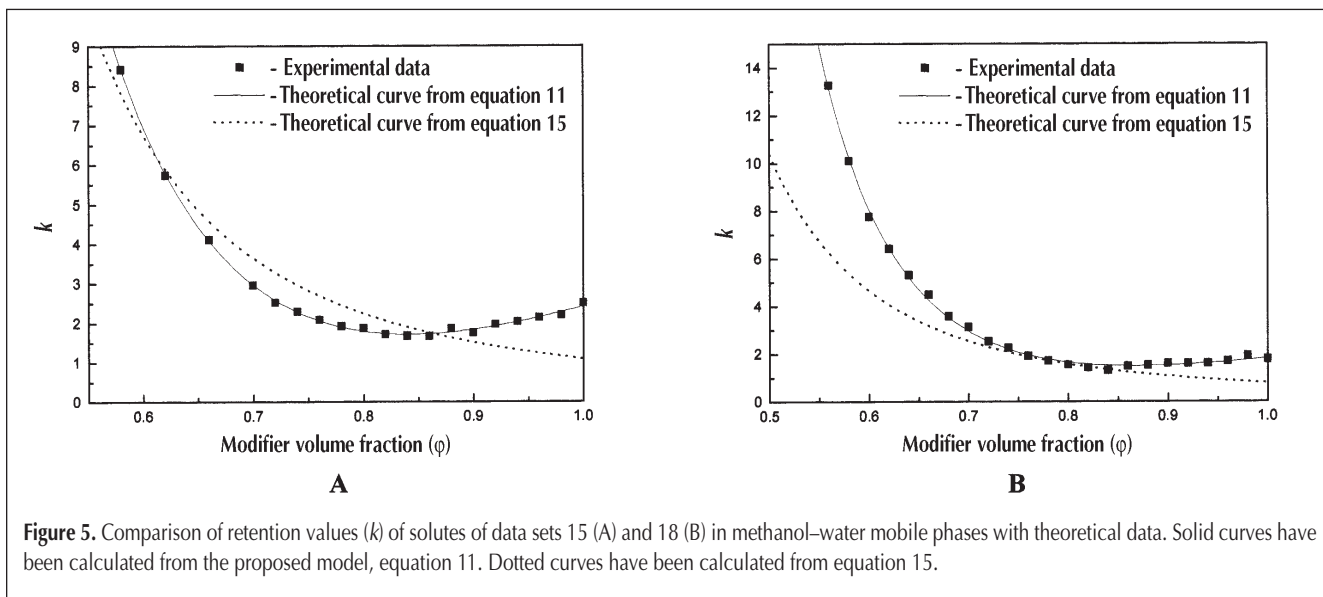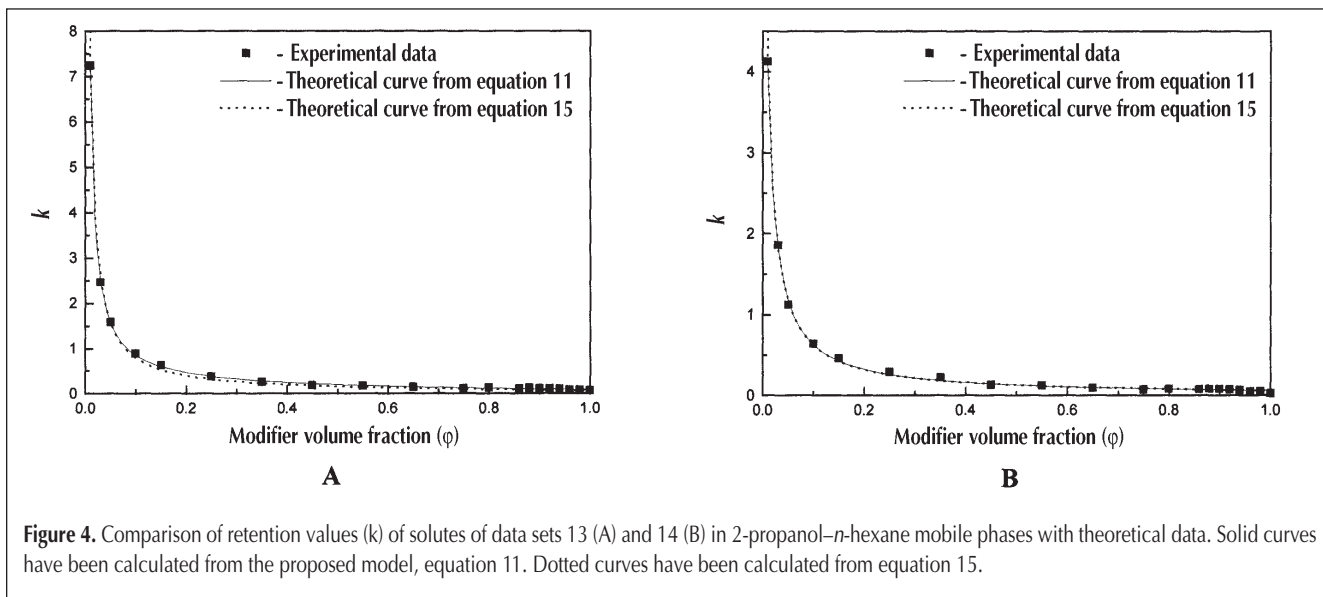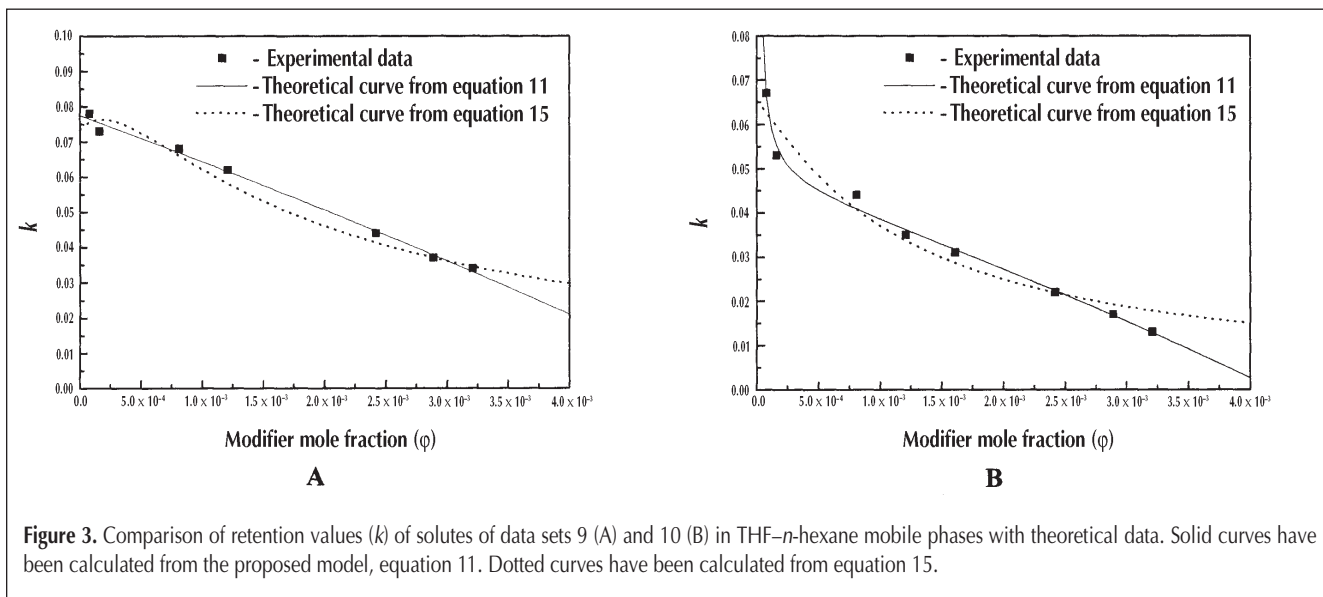
Eq. 15

Both models were compared in different HPLC systems presented in Table I by means of three statistical criteria (equations 12–14). Table II also specifies the values of the sums of the squared differences between experimental and theoretical data, SDs, and the Fisher test values obtained as a results of the comparison between the Nikitas–Pappa-Louisi model (equation 15) and the experimental data. In Figures 1–5, dotted lines show the theoretical curves obtained from equation 15. On the basis of the comparison of the three statistical criteria for both models (equations 11 and 15), it can be concluded that the newly proposed model (equation 11) gives some better-fitting results and accuracy than equation 15, which was proposed by Nikitas et al. (9,13). In particular, the best results of computations for a proposed adsorption model (equation 11) were obtained in the case of retention data at low levels of the organic modifiers (data sets 9–12, Figure 3) and crown compounds (data sets 15–18, Figure 5).

Conclusion

From the study reported here, the following conclusions can be drawn: a new adsorption 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 the use of many different analytes, columns, and sorbents with chemically bonded ligands (only selected results are presented in this study). All the computation results obtained confirm very good performance of the proposed model (equation 11). This model gives good fitting results, accuracy, and great applicability.

Figure 1. Comparison of retention values (k) of solutes of data sets 2 (A) and 4 (B) in methanol–water mobile phases with theoretical data. Solid curves have been calculated from the proposed model, equation 11. Dotted curves have been calculated from equation 15.

Figure 2. Comparison of retention values (k) of solutes of data set 6 (A) in isopropanol–water and of data set 8 (B) in acetonitrile–water mobile phase with theoretical data. Solid curves have been calculated from the proposed model, equation 11. Dotted curves have been calculated from equation 15.

Figure 3. Comparison of retention values (*k*) of solutes of data sets 9 (A) and 10 (B) in THF–*n*-hexane mobile phases with theoretical data. Solid curves have been calculated from the proposed model, equation 11. Dotted curves have been calculated from equation 15.

Figure 4. Comparison of retention values (k) of solutes of data sets 13 (A) and 14 (B) in 2-propanol–*n*-hexane mobile phases with theoretical data. Solid curves have been calculated from the proposed model, equation 11. Dotted curves have been calculated from equation 15.

Figure 5. Comparison of retention values (*k*) of solutes of data sets 15 (A) and 18 (B) in methanol–water mobile phases with theoretical data. Solid curves have been calculated from the proposed model, equation 11. Dotted curves have been calculated from equation 15.

Table II. Estimated Numerical Values of Equation 11 Parameters and Values of Statistical Criteria with Aid of Equations 11 and 15

| Data | Equation 11 | | | | | | | Equation 15 | | |
	p_1	p_2	p_3	p_4	SUM	SD	F	SUM	SD	F
1	20.91 ± 17.8	-0.63 ± 0.13	-0.43 ± 0.08	-5.47 ± 1.10	0.3198	0.2137	763.3	0.2320	0.1701	1205.0
2	2.22 ± 1.42	-0.52 ± 0.16	-0.50 ± 0.10	-5.28 ± 0.58	0.0027	0.0197	2275.0	0.0032	0.0201	2178.0
3	10.35 ± 8.01	-0.79 ± 0.13	-0.37 ± 0.08	-4.84 ± 0.53	0.0026	0.0297	1.15×10^4	0.0013	0.0183	3.0×10^4
4	2.74 ± 0.36	-0.45 ± 0.06	0.60 ± 0.3	-5.14 ± 0.56	0.0004	0.0109	2.46×10^4	0.0018	0.0211	6543.0
5	$4.54 \times 10^{-4} \pm 2 \times 10^{-4}$	$14,390 \pm 1000$	-836.2 ± 121	-4.58 ± 1.23	3.917	1.1430	54.8	5.1820	1.1380	55.25
6	40.03 ± 24.50	-1.71 ± 1.10	-7.29×10^{-4}	222.4 ± 160.0	0.0502	0.1121	2738.0	0.3223	0.2318	1539.0
7	315.3 ± 130.9	-0.57 ± 0.02	-0.45 ± 0.01	-3.29 ± 0.05	0.0460	0.0811	2.11×10^4	0.0608	0.0872	1.8×10^4
8	30.18 ± 1.24	-0.89 ± 0.07	-0.39 ± 0.06	-3.99 ± 0.06	0.0385	0.1133	7.69×10^4	9.6440	1.5530	406.9
9	0.08 ± 0.004	-189.10 ± 6.40	-24.79 ± 1.36	0	1.16×10^{-5}	0.0017	111.40	8.5×10^{-5}	0.0041	17.36
10	0.05 ± 0.008	-240.2 ± 8.40	$3.46 \times 10^{-5} \pm 3.3 \times 10^{-6}$	-40.64 ± 2.82	1.77×10^{-5}	0.0021	78.16	1.1×10^{-4}	0.0047	15.78
11	0.11 ± 0.02	$6.09 \times 10^{-7} \pm 1.0 \times 10^{-7}$	169.8 ± 49.5	1143.0 ± 139.5	0.0001	0.0053	22.82	0.0002	0.0060	17.65
12	0.08 ± 0.01	-53.69 ± 11.6	$3.45 \times 10^{-5} \pm 1.0 \times 10^{-5}$	674.0 ± 51.8	9.22×10^{-6}	0.0015	478.50	9.2×10^{-5}	0.0043	60.20
13	0.35 ± 0.04	-0.39 ± 0.054	0.19 ± 0.021	2.31 ± 0.40	0.0102	0.0253	4235.0	0.0429	0.0502	1073.0
14	7.58 ± 1.10	-0.08 ± 0.01	0.0015 ± 0.001	111.2 ± 17.3	0.0105	0.0256	1434.0	0.0106	0.0256	1499.0
15	217.3 ± 15.72	-0.63 ± 0.007	-0.42 ± 0.003	-5.61 ± 2.85	0.0530	0.0594	819.0	7.7440	0.6957	5.98
16	25.10 ± 8.34	-0.65 ± 0.01	-0.43 ± 0.005	-2.52 ± 0.30	0.0163	0.0331	1144.0	1.5460	0.3211	10.0
17	44.83 ± 6.30	-0.62 ± 0.05	-0.45 ± 0.02	-2.43 ± 1.10	0.2238	0.1312	95.74	2.3090	0.4061	9.994

The model (equation 11) was compared with the literature-known adsorption model (equation 15) developed by Nikitas et al. The results of this comparisons show that, especially in the strong parabolic $k = f(\varphi)$ dependence (for crown compounds), the newly proposed adsorption model (equation 11) gives the most accurate results of computation. The usefulness of equation 11 will be further tested in the future by the modeling of different retention data in TLC.

References

1. P.J. Schoenmakers. Optimization of chromatographic selectivity. A guide to method development. *J. Chromatogr. Library,* **35**. Elsevier, Amsterdam, the Netherlands, 1986.
2. K. Kaczmarski, W. Prus, and T. Kowalska. A new adsorption-partition model of solute retention in chromatographic systems with chemically bonded stationary phases. *J. Planar Chromatogr.* **12:** 175–79 (1999).
3. K.H. Row. Comparison of retention models for the dependence of retention factors on mobile phase compositions in reversed-phase high performance liquid chromatography. *J. Chromatogr. A* **797:** 23–31 (1998).
4. Y.W. Lee, K.H. Row, M.S. So, I.A. Polunina, and A.V. Larin. Reversed-phase HPLC retention of deoxyribonucleosides as a function of mobile phase composition. *J. Liq. Chromatogr.* **18(15):** 3077–89 (1995).
5. K. Kaczmarski, W. Prus, and T. Kowalska. Adsorption/partition model of liquid chromatography for chemically bonded stationary phases of the aliphatic cyano, reversed-phase C8 and reversed-phase C_{18} types. *J. Chromatogr. A* **869:** 57–64 (2000).
6. W. Zapala, K. Kaczmarski, and T. Kowalska. Comparison of different retention models in normal and reversed-phase liquid chromatography with binary mobile phases. *J. Chromatogr. Sci.* **40:** 575–80 (2002).
7. Y.D. Kahie, C. Pietrogrande, M.I. Medina Mendez, P. Reschiglian, and F. Dondi. Effects of stationary phase structure on retention and selectivity in reversed-phase liquid chromatography of flavonoid compounds with methanol as organic modifier. *Chromatographia* **30(7/8):** 447–52 (1990).
8. S.N. Lanin, M.Y. Ledenkova, and Y.S. Nikitin. Influence of the concentration of adsorbate and modifier in the mobile phase on retention in high-performance liquid chromatography. *J. Chromatogr. A* **797:** 3–9 (1998).
9. P. Nikitas, A. Pappa-Louisi, and P. Agrafiotou. Effect of the organic modifier concentration on the retention in reversed-phase liquid chromatography. I. General semi-thermodynamic treatment for adsorption and partition mechanisms. *J. Chromatogr. A* **946:** 9–32 (2002).
10. G. Guiochon, S.G. Shirazi, and A.M. Katti. *Fundamentals of Preparative and Nonlinear Chromatography.* Academic Press, Boston, MA, 1994.
11. R. Fletcher. A modified Marquardt sub-routine for nonlinear least-squares. Report AERE-R6799, Atomic Energy Research Establishment, Harwell, England, 1971.
12. G.A.F. Seber and C.J. Wild. *Nonlinear Regression.* Wiley, New York, NY, 1989.
13. P. Nikitas and A. Pappa-Louisi. New equations describing the combined effect of pH and organic modifier concentration on the retention in reversed-phase liquid chromatography. *J. Chromatogr. A* **971:** 47–60 (2002).