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New equations describing the combined effect of pH and organic modifier concentration on the retention in reversed-phase liquid chromatography

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Abstract

Six equations that express the combined effect of mobile phase pH and organic modifier content on sample retention in reversed-phase liquid chromatography (RPLC) are developed based on either the adsorption or the partition model for retention. The equations are tested against five retention data sets taken from literature. In the tests two pH scales are used, ${}^{w}_{w}$pH and ${}^{s}_{s}$pH. It is shown that a new seven-parameter equation works more satisfactorily, because it exhibits good numerical behavior, gives low values of the sum of squares of residuals and represents the experimental retention surfaces successfully. In addition, the danger of overfitting, which leads to the prediction of physically meaningless retention surfaces, is minimized by using the proposed new seven-parameter equation. Finally, the possibility of obtaining reliable pK values of weak acids or bases chromatographically by means of the derived equations is also considered and discussed.
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1. Introduction

In a recent paper [1] we presented and tested against a variety of experimental data ten simplified expressions for the retention factor k of a solute as a function of the volume fraction φ of the organic modifier in the mobile phase. These expressions arise from either the adsorption or the partition model for retention in reversed-phase chromatographic columns [2–5]. The study showed that a new three-parameter expression of ln k, developed within the frames of the adsorption model, gives the best fitting results, since it combines simplicity, accuracy, good numerical behavior and great applicability. This expression of ln k may be written as:

$$\ln k = a - \ln(1 + b\varphi) - \frac{\varphi c}{1 + b\varphi} \quad (1)$$

where a, b and c are constants, from which b is related to the free energy of adsorption of the organic modifier on the stationary phase.

Eq. (1) as well as all equations examined in [1] are valid at constant pH. Therefore, it would be of great interest to apply the above equation to the development of equations that describe the combined effect of φ and pH on the retention factor of eluted

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species that are either weak acids or bases. This is attempted in the present communication. Note that the present investigation was motivated not only by the fact that we have developed Eq. (1), which works better than any other equation, but also because we have found that the conventional equation usually used to describe the combined effect of φ and pH on the retention factor of weak acids or bases [6,7] may predict physically meaningless retention surfaces [8]. Thus, there is need for the development of better models for the dependence of k upon both the mobile phase pH and organic modifier content.

2. Basic equations describing the effect of pH on the retention factor

When a weak monoprotic acid AH is eluted from a mobile phase consisting of the solvent S and the organic modifier B, the basic equation for the retention factor may be written as [4,9,10]:

$$k = \lim_{n^{m}_{AH}, n^{m}_{A^-} \to 0} \frac{n^{s}_{AH} + n^{s}_{A^-}}{n^{m}_{AH} + n^{m}_{A^-}} \tag{2}$$

where n^{s}_{AH}, $n^{s}_{A^-}$, n^{m}_{AH}, $n^{m}_{A^-}$ are the numbers of moles of the acid AH and its conjugated base A^- at the stationary phase (s) and in the mobile phase (m) inside the chromatographic column, respectively. This equation can be written in terms of mole fractions if we adopt the lattice model approximation to describe the properties of the mobile phase, i.e. if we assume that the mobile phase has a lattice structure where each molecule occupies one site [2]. Then taking into account that n^{m}_{AH}, $n^{m}_{A^-} \to 0$, we readily obtain:

$$k = k^{*} \frac{x^{s}_{AH} + x^{s}_{A^-}}{x^{m}_{AH} + x^{m}_{A^-}} \tag{3}$$

where $k^{*} = (n^{s}_{B} + n^{s}_{S})/(n^{m}_{B} + n^{m}_{S})$. Eq. (3) can be rearranged as:

$$k = k_{AH} \frac{x^{m}_{AH}}{x^{m}_{AH} + x^{m}_{A^-}} + k_{A^-} \frac{x^{m}_{A^-}}{x^{m}_{AH} + x^{m}_{A^-}} \tag{4}$$

where

$$k_{AH} = k^{*} x^{s}_{AH} / x^{m}_{AH} \quad \text{and} \quad k_{A^-} = k^{*} x^{s}_{A^-} / x^{m}_{A^-} \tag{5}$$

From the equilibrium $AH \leftrightarrow A^- + H^+$ in the mobile phase, we obtain:

$$K_a = \frac{a^{m}_{A^-} a^{m}_{H^+}}{a^{m}_{AH}} = a^{m}_{H^+} \frac{x^{m}_{A^-}}{x^{m}_{AH}} \frac{\gamma^{m}_{A^-}}{\gamma^{m}_{AH}} \tag{6}$$

where a_i and γ_i denote the activity and the activity coefficient of species i, respectively. Substitution of the ratio $x^{m}_{A^-}/x^{m}_{AH}$ from Eq. (6) into Eq. (4) yields:

$$k = \frac{k_0 + k_1 10^{pH-pK} \gamma^{m}_{0} / \gamma^{m}_{1}}{1 + 10^{pH-pK} \gamma^{m}_{0} / \gamma^{m}_{1}} \tag{7}$$

where for simplicity K denotes K_a, subscript (0) stands for AH and subscript (1) for A^-. If the eluted species is a weak monoprotic basis BH^+ ($BH^+ \leftrightarrow B + H^+$), then Eq. (7) is still valid, but now (0) denotes BH^+ and (1) stands for B.

Eq. (7) describes the effect of pH on the retention factor k of a monionogenic solute. Variations of this equation can be found in literature. Thus Eq. (7) without the ratio of the activity coefficients has been derived by Schoenmakers et al. [6,7], whereas recently Rosés et al. [11] have proved Eq. (7) without the activity coefficient of AH, γ^{m}_{0}. We should point out that in pure aqueous solutions and in the limit $n^{m}_{AH} \to 0$ the activity coefficient of the uncharged species AH, γ^{m}_{0}, tends to unity and therefore it can be eliminated. However, this is no more valid when the mobile phase is a mixture of the solvent S (aqueous buffer) and the organic modifier B. In this case the activity coefficient γ^{m}_{0} as well as γ^{m}_{1} depend upon φ [1,2,12,13] and this dependence should be taken into account when Eq. (7) is used in mobile phases containing an organic modifier. Note that in Eq. (7) the factor $10^{-pK} \gamma^{m}_{0} / \gamma^{m}_{1}$ can be always written as $10^{-pK^{*}}$, where $pK^{*} = pK - \log \gamma^{m}_{0} + \log \gamma^{m}_{1}$. Therefore, there is a problem of whether the variation of pK upon φ in Eq. (7) can be distinguished or not from the corresponding variation of the ratio $\gamma^{m}_{0} / \gamma^{m}_{1}$ also from φ. This problem is addressed below.

3. The modifier effect

Eq. (7) can be extended to describe the combined effect of mobile phase pH and modifier content on the retention of an ionogenic solute if k_0, k_1, pK and

the ratio γ_0^m/γ_1^m are expressed in terms of φ. Eq. (1) developed in paper [1] on the basis of the adsorption model can describe the variation of retention of each species with organic content at a given pH value. This equation can be written as:

$$\ln k_i = \ln k_i^0 - \ln(1 + b\varphi) - \frac{c_i\varphi}{1 + b\varphi},$$
$$i = 0 \text{ or } 1 \quad (8)$$

Alternatively, $\ln k_i$ may be calculated from the most commonly used equation:

$$\ln k_i = \ln k_i^0 + s_i\varphi + t_i\varphi^2, \quad i = 0 \text{ or } 1 \quad (9)$$

which is based on the partition model [1–4].

The dependence of pK upon the composition of the mobile phase may be derived from Born's equation, which in the case of an acid–base equilibrium yields [14,15]:

$$\mathrm{pK} = \mathrm{pK}_r + \frac{D}{\epsilon} \quad (10)$$

where pK_r and D are constants and ϵ is the dielectric constant of the mobile phase. In fact Born's equation leads to a more complicated equation [16,17] and thus Eq. (10) should be considered as an approximation.

The dielectric constant of the mobile phase can be easily measured by a dipolmeter. If this is not possible, we may take into account that ϵ and/or $1/\epsilon$ depend linearly on φ provided that the range of the φ values is not wide enough, otherwise this dependence is quadratic. Thus in general we have:

(a) If $\epsilon = \epsilon_0 + \epsilon_1\varphi + \epsilon_2\varphi^2$, then:

$$\mathrm{pK} = \mathrm{pK}_r + 1/(e_0 + e_1\varphi + e_2\varphi^2) \quad (11)$$

where $e_i = \epsilon_i/D$.

(b) If $1/\epsilon = \epsilon_0' + \epsilon_1'\varphi + \epsilon_2'\varphi^2$, then:

$$\mathrm{pK} = \mathrm{pK}^0 + r_1\varphi + r_2\varphi^2 \quad (12)$$

which readily yields:

$$\ln K = \ln K^0 + q_1\varphi + q_2\varphi^2 \quad (13)$$

Here, $\mathrm{pK}^0 = \mathrm{pK}_r + D\epsilon_0'$, $r_i = D\epsilon_i'$ and $q_i = -r_i \ln 10$. Note that pK^0 is the value of pK at $\varphi = 0$ and pK_r is related to pK^0 via the equation $\mathrm{pK}_r = \mathrm{pK}^0 - 1/e_0$. It is also interesting to note that Eq. (13) has already been used rather empirically by Marques and Schoenmakers [7], without any connection to Born's equation.

Finally, the dependence of the activity coefficients γ_0^m, γ_1^m on φ may be determined by the treatment presented in [1,2] using a straightforward extension in order to include two eluted species AH and A^- or BH^+ and B. If we take the first six terms in the expression of the excess free energy G^e of a quaternary mixture containing AH, A^-, (or BH^+, B), organic modifier and solvent, then we obtain:

$$\ln \gamma_0^m = F_0 + F_1\varphi + F_2\varphi^2 \quad \text{and}$$
$$\ln \gamma_1^m = D_0 + D_1\varphi + F_2\varphi^2 \quad (14)$$

Note that the coefficients of the last term of Eqs. (14) are identical. Thus the ratio γ_0^m/γ_1^m may be expressed as:

$$\gamma_0^m/\gamma_1^m = e^{g_0 + g_1\varphi} \quad (15)$$

where $g_0 = F_0 - D_0$ and $g_1 = F_1 - D_1$.

At this point it is worth noting, relatively to the solute standard states, the following: Consider a ternary solution composed of the solvent S, the solute A and the organic modifier B with a volume fraction φ. The chemical potential of A may be expressed as $\mu_A = \mu_A^0 + RT \ln f_A x_A$, where μ_A^0 is the standard chemical potential of A and f_A is the activity coefficient of A. One possible choice for the standard states is the asymmetrical choice, for which we have $f_A \to 1$ when $x_A \to 0$ [18]. This standard state is totally fictitious and μ_A^0 is the chemical potential of A in the limit $x_A = 1$ of a hypothetical solution that retains the ideal properties of the dilute state up to the pure state of A and the volume fraction φ of the modifier B in the solvent S remains constant. Thus μ_A^0 is in fact a function of φ, $\mu_A^0(\varphi)$, and therefore in the limit $x_A \to 0$ the chemical potential of A may be expressed as $\mu_A = \mu_A^0(\varphi) + RT \ln x_A$. However, $\mu_A^0(\varphi)$ can be always written as $\mu_A^0(\varphi) = \mu_A^0 + RT \ln f_A(\varphi)$, where $f_A(\varphi)$ is a function of φ. Therefore, the chemical potential of A in the limit $x_A \to 0$ may be alternatively expressed as $\mu_A = \mu_A^0 + RT \ln f_A(\varphi)x_A$, where μ_A^0 is the value of $\mu_A^0(\varphi)$ in the limit $\varphi \to 0$. It is seen that in this expression of the chemical potential of A the activity coefficient f_A is no more equal to unity in the limit $x_A \to 0$ but it depends upon

φ. This dependence is expressed by Eqs. (14) presented above.

4. Equations describing the combined effect of pH and the organic modifier content on the retention factor

The final equations for the combined effect of pH and φ on the retention factor k arise from the substitution of Eqs. (8)–(15) into Eq. (7). We obtain the following general expressions for k:

(a) Substitution of Eqs. (9), (13) and (15) into Eq. (7) yields:

$$k = \frac{k_0^0\, \mathrm{e}^{s_0\varphi + t_0\varphi^2} + k_1^0 K^* \mathrm{e}^{h_1\varphi + h_2\varphi^2} 10^{\mathrm{pH}}}{1 + K^* \mathrm{e}^{p_1\varphi + p_2\varphi^2} 10^{\mathrm{pH}}} \tag{16}$$

where $K^* = K^0\ \mathrm{e}^{g_0}$, $h_1 = s_1 + g_1 + q_1$, $h_2 = t_1 + q_2$, $p_1 = g_1 + q_1$ and $p_2 = q_2$. If ϵ varies linearly with φ, then $q_2 = p_2 = 0$. Note that this equation has been previously derived from Marques and Schoenmakers [7] following a different approach.

(b) If Eqs. (9), (10) and (15) are substituted into Eq. (7), we obtain:

$$k = \frac{k_0^0\, \mathrm{e}^{s_0\varphi + t_0\varphi^2} + k_1^0 K_r^* 10^{\mathrm{pH} - D/\epsilon}\ \mathrm{e}^{h_1\varphi + h_2\varphi^2}}{1 + K_r^* 10^{\mathrm{pH} - D/\epsilon}\ \mathrm{e}^{p_1\varphi}} \tag{17}$$

where here $K_r^* = K_r\ \mathrm{e}^{g_0}$, $h_1 = s_1 + g_1$, $h_2 = t_2$ and $p_1 = g_1$. Note that the physical meaning of the various coefficients of Eq. (17) may be slightly different from that of Eq. (16). However, we have adopted the same symbols for simplicity.

(c) If Eq. (11) is used instead of Eq. (10), then Eq. (17) is written as:

$$k = \frac{k_0^0\, \mathrm{e}^{s_0\varphi + t_0\varphi^2} + k_1^0 K_r^* 10^{\mathrm{pH} - 1/(e_0 + e_1\varphi + e_2\varphi^2)}\ \mathrm{e}^{h_1\varphi + h_2\varphi^2}}{1 + K_r^* 10^{\mathrm{pH} - 1/(e_0 + e_1\varphi + e_2\varphi^2)}\ \mathrm{e}^{p_1\varphi}} \tag{18}$$

Eqs. (16), (17) and (18) are based on the partition model of retention. Three other general equations arise from the adsorption model and they may be expressed as follows:

(d)

$$k = \frac{k_0^0 \exp\left(\dfrac{-\varphi c_0}{1 + b\varphi}\right) + k_1^0 K^* 10^{\mathrm{pH}}\ \mathrm{e}^{h_1\varphi + h_2\varphi^2 - \frac{\varphi c_1}{(1 + b\varphi)}}}{(1 + b\varphi)(K^* 10^{\mathrm{pH}}\ \mathrm{e}^{h_1\varphi + h_2\varphi^2} + 1)} \tag{19}$$

where $h_1 = q_1 + g_1$ and $h_2 = q_2$.

(e)

$$k = \frac{k_0^0 \exp\left(\dfrac{-\varphi c_0}{1 + b\varphi}\right) + k_1^0 K_r^* 10^{\mathrm{pH} - D/\epsilon}\ \mathrm{e}^{h_1\varphi - \frac{\varphi c_1}{(1 + b\varphi)}}}{(1 + b\varphi)(K_r^* 10^{\mathrm{pH} - D/\epsilon}\ \mathrm{e}^{h_1\varphi} + 1)} \tag{20}$$

where $h_1 = g_1$.

(f)

$$k = \frac{k_0^0 \exp\left(\dfrac{-\varphi c_0}{1 + b\varphi}\right) + k_1^0 K_r^* 10^{\mathrm{pH} - 1/(e_0 + e_1\varphi + e_2\varphi^2)}\ \mathrm{e}^{h_1\varphi - \frac{\varphi c_1}{(1 + b\varphi)}}}{(1 + b\varphi)(K_r^* 10^{\mathrm{pH} - 1/(e_0 + e_1\varphi + e_2\varphi^2)}\ \mathrm{e}^{h_1\varphi} + 1)}. \tag{21}$$

Eq. (19) is derived from substitution of Eqs. (8), (13) and (15) into Eq. (7), Eq. (20) results if Eq. (13) is replaced by Eq. (10), whereas the use of Eq. (11) instead of Eq. (10) results in Eq. (21). Note also that in Eqs. (17) and (20) K_r^* can be replaced by K^* provided that D/ϵ will be replaced by $D(1/\epsilon - 1/\epsilon_0)$, where ϵ_0 is the dielectric constant in the limit $\varphi = 0$.

5. Results and discussion

The performance of all the above equations was tested using five retention data sets taken from the literature [19–23]. Their basic features are shown in Table 1. All systems contain enough data points for a reliable fitting, except perhaps the system of hydroxizine in methanol–aqueous buffers, which has been included in our study for the following reason. When the retention modelling is used for optimization of a separation, it is desirable to find out a proper model on the basis of a limited number of experimental data points. Marques and Schoenmakers [7,19] have found that a 4×3 experimental design covering 4 units of pH (pH 4, 5, 6 and 7) is appropriate for this

Table 1
Experimental systems used

System	Solute	Mobile phase	Column	φ range	pH range	Data points	Ref.
1	Hydroxizine	Methanol–water	Chromspher C_{18}	0.65–0.75	4–7	12	[19]
2	Benzoic acid	Methanol–water	Chromspher C_{18}	0.30–0.55	2.6–7	36	[20]
3	Adenosine	Acetonitrile–water	PRP-1 Polymeric	0–0.10	2.1–6.9	28	[21]
4	Adenosine	Methanol–water	PRP-1 Polymeric	0–0.15	2.1–6.9	31	[21]
5	5-hydroxyindole-3-acetic acid	Isopropanol–water	Inertsil ODS-3	0–0.05	3.1–7.74	30	[23]

purpose. The system of hydroxizine fulfills the above requirement. An additional requirement for a reliable fitting is that the range of pH values used should be broad enough including the pK_a of the analyte. This condition is also fulfilled in the systems we studied, since the pK_a values of the analytes are the following: 5.3 for hydroxizine, measured chromatographically in 70% v/v methanol–water solutions [6,19]; 4.2 for benzoic acid in water [24]; 3.5 for adenosine in water [25]; 4.4 for 5-hydroxyindole-3-acetic acid in water, measured potentiometrically in the present study.

In all data sets adopted in the present study the pH has been measured in the aqueous buffer before mixing it with the organic modifier. This pH scale denoted by ${}^{w}_{w}$pH [17,26] has been adopted in the vast majority of the relevant studies, because it is very simple, does not present technical difficulties in automated systems or routine optimization procedures and reduces the required number of pH measurements [7]. However, the rigorous thermodynamic pH scale in mobile phases modified by an organic solvent is defined from ${}^{s}_{s}$pH $= -\log a_H$, where a_H is the hydrogen ion activity in the mobile phase. This pH scale is related to the ${}^{s}_{w}$pH scale, i.e. when the pH is measured after mixing the aqueous buffer with the organic modifier using a pH electrode system calibrated with common aqueous reference buffers, by the following relationship [17,26,27]:

$$ {}^{s}_{s}\mathrm{pH} = {}^{s}_{w}\mathrm{pH} - \delta \qquad (22) $$

where the quantity δ depends only on the composition of the mobile phase. For modifier contents less than 15% the term δ has a negligible contribution [17,26,27]. This term has a small but detectable contribution for methanol contents above 30% (v/v) in the systems of hydroxizine and benzoic acid.

In the present study we used both ${}^{w}_{w}$pH and ${}^{s}_{s}$pH scales. The latter scale has been obtained by means of Eq. (22) using tabulated δ values only for methanol contents above 30% (v/v) [26,27]. The ${}^{s}_{w}$pH measurements were carried out using a Beckman Research pH meter. The electrode system used was a Beckman 39004, type E-2, glass electrode coupled with an Ingold, type 303-NS-EK, saturated calomel electrode. Two aqueous buffer solutions were used to standardize the pH values: (a) 0.05 m KH phthalate with pH = 4.008, and (b) 0.025 m KH_2PO_4 + 0.025 m Na_2HPO_4 with pH = 6.865. Temperature was controlled at 25 °C and a nitrogen atmosphere was maintained above the working solution in an experimental set up described in [27].

The empirical relationships found between the ${}^{w}_{w}$pH and ${}^{s}_{w}$pH scales are given in Table 2. These relationships are valid within the pH and φ ranges shown in Table 1. Table 2 depicts also the dependence of the dielectric constant on φ, which is necessary for testing Eqs. (17) and (20). The dielectric constants were measured by means of a dipolmeter type DM 01.

The whole treatment was carried out on Microsoft

Table 2
Dependence of the dielectric constant ϵ on φ, and ${}^{s}_{w}$pH on φ and ${}^{w}_{w}$pH

System	ϵ dependence on φ	${}^{s}_{w}$pH dependence on φ and ${}^{w}_{w}$pH
1	$\epsilon = -49.0\varphi + 85.2$	${}^{s}_{w}\mathrm{pH} = 1.1905\varphi^2 + 0.0684\ {}^{w}_{w}\mathrm{pH}\varphi + 0.6439\varphi + 1.0162\ {}^{w}_{w}\mathrm{pH}$
2	$\epsilon = -38.86\varphi + 79.28$	${}^{s}_{w}\mathrm{pH} = 1.1905\varphi^2 + 0.0684\ {}^{w}_{w}\mathrm{pH}\varphi + 0.6439\varphi + 1.0162\ {}^{w}_{w}\mathrm{pH}$
3	$\epsilon = -26.09\varphi + 78.51$	${}^{s}_{w}\mathrm{pH} = 0.1627\ {}^{w}_{w}\mathrm{pH}\varphi + 1.5624\varphi + {}^{w}_{w}\mathrm{pH}$
4	$\epsilon = -35.33\varphi + 78.54$	${}^{s}_{w}\mathrm{pH} = 0.1723\ {}^{w}_{w}\mathrm{pH}\varphi + 0.726\varphi + {}^{w}_{w}\mathrm{pH}$
5	$\epsilon = -51.11\varphi + 78.52$	${}^{s}_{w}\mathrm{pH} = 0.2385\ {}^{w}_{w}\mathrm{pH}\varphi + 0.5915\varphi + {}^{w}_{w}\mathrm{pH}$

Table 3
Fitted parameters for Eqs. (16)–(17) using ${}^{w}_{w}$pH values

Eq.:	16(9)[a]	16(8)	16(r)[b]	17(9)	17(8)	17(r)
Hydroxizine in methanol–water buffers						
k_0^0	17.6±0.7	17.6±0.6	17.8±0.4	17.6±0.7	17.6±0.6	17.8±0.4
k_1^0	7.0±0.6	7.0±0.5	7.1±0.4	7.0±0.6	7.0±0.5	7.1±0.4
pK*	5.35±0.16	5.36±0.13	5.32±0.09	5.35±0.15	5.36±0.13	5.32±0.08
h_1	−11.6±28	−7.8±1.9	−11.3±1.2	−25.1±118	−11.2±4.6	−11.3±1.2
h_2	48.6±305	4.7±51	–	10.8±72	6.0±51	–
s_0	−8.1±2.9	−7.8±1.9	−9.0±0.5	−8.1±2.8	−7.8±1.9	−9.0±0.5
t_0	−8.1±32	−11.3±21	–	−8.1±32	−10.7±21	–
p_1	0.1±23	3.4±6.5	–	−13.4±114	–	–
p_2	37.8±256	–	–	–	–	–
D	–	–	–	−396±2682	−82.1±154	–
χ^2	1.009	1.015	1.156	1.009	1.012	1.156
σ^2	0.336	0.254	0.165	0.336	0.253	0.165
Benzoic acid in methanol–water buffers						
k_0^0	5.44±0.03	5.44±0.03	5.44±0.03	5.44±0.03	5.44±0.03	5.44±0.03
k_1^0	0.22±0.02	0.22±0.02	0.22±0.02	0.22±0.02	0.22±0.02	0.22±0.02
pK*	4.26±0.01	4.26±0.01	4.26±0.01	4.26±0.01	4.26±0.01	4.26±0.01
h_1	117±33	118±31	118±31	113±42	122±31	122±31
h_2	−1278±338	−1293±311	−1293±311	−1278±336	−1305±307	−1305±307
s_0	−7.4±0.25	−7.4±0.2	−7.4±0.2	−7.4±0.2	−7.4±0.2	−7.4±0.2
t_0	1.7±1.4	1.8±1.1	1.8±1.1	1.7±1.3	1.9±1.1	1.9±1.1
p_1	−2.6±1.4	−2.8±0.5	−2.8±0.5	−6.1±19	–	–
p_2	−1.3±8.3	–	–	–	–	–
D	–	–	–	−149±870	125±20	125±20
χ^2	0.063	0.063	0.063	0.063	0.063	0.063
σ^2	0.0023	0.0022	0.0022	0.0023	0.0022	0.0022
Adenosine in acetonitrile–water buffers						
k_0^0	0.93±0.37	0.99±0.49	0.69±0.16	1.07±0.48	0.98±0.48	1.11±0.41
k_1^0	22.6±0.3	22.4±0.3	22.5±0.3	22.5±0.3	22.4±0.3	22.5±0.3
pK*	3.48±0.06	3.48±0.07	3.45±0.04	3.48±0.07	3.47±0.07	3.48±0.06
h_1	−57.0±20	−78.9±23	−55.0±18	204±202	−72.7±2.8	193±192
h_2	−659±370	421±48	−731±350	417±47	419±48	417±47
s_0	−9.8±21	−12.4±54	–	−10.5±52	−7.8±51	−20.9±21
t_0	42.7±220	−30.3±1000	−24.9±19	−243±1054	−106±958	–
p_1	22.8±21	−6.1±24	−1439±376	277±202	–	265±193
p_2	−1366±400	–	–	–	–	–
D	–	–	–	27 441±19 302	930±2260	26 172±18 276
χ^2	3.500	4.846	3.6120	4.402	4.822	4.415
σ^2	0.184	0.242	0.172	0.232	0.241	0.221
Adenosine in methanol–water buffers						
k_0^0	1.17±0.55	1.21±0.55	0.79±0.24	1.18±0.60	1.22±0.55	1.19±0.37
k_1^0	22.3±0.4	22.4±0.4	22.3±0.4	22.4±0.4	22.4±0.4	22.4±0.4
pK*	3.49±0.08	3.50±0.08	3.46±0.05	3.49±0.09	3.50±.08	3.49±0.07
h_1	−33.7±13	−24.6±8	−24.6±1	−117±110	−24.5±1.1	−114±77
h_2	185±115	82.3±10	82.5±9	80.2±13	82.4±10	80.2±10
s_0	1.96±25	−8.1±19	–	0.98±25	−8.8±19	–
t_0	–99±257	15.7±129	–	−90.2±263	17.7±119	−82.1±105
p_1	−9.5±14	−0.1±8	–	−92.5±111	–	90.0±77
p_2	106±118	–	–	–	–	–
D	–	–	–	−6832±8025	−31.2±597	−6655±5663
χ^2	7.824	8.070	8.477	7.797	8.070	7.798
σ^2	0.356	0.351	0.326	0.354	0.351	0.339

Table 3. Continued

Eq.:	16(9)[a]	16(8)	16(r)[b]	17(9)	17(8)	17(r)
5-hydroxyindole-3-acetic acid in isopropanol–water buffers						
k_0^0	119.7±2.4	119.1±2.5	119.7±2.4	119.2±2.4	119.1±2.5	119.2±2.4
k_1^0	11.0±1.6	11.0±1.7	11.0±1.6	10.8±1.6	11.0±1.7	10.8±1.6
pK*	4.38±0.05	4.39±0.05	4.38±0.05	4.40±0.05	4.39±0.05	4.40±0.05
h_1	−135±60	−89.8±47	−135±60	888±488	−91.8±36	888±488
h_2	4045±1340	954±939	4045±1340	775±683	966±952	775±683
s_0	−141±9	−125±6	−141±9	−130±7	−125±6	−130±7
t_0	2473±389	1446±161	2473±389	1838±293	1445±161	1838±293
p_1	−43.1±38	1.0±22	−43.1±38	962±494	–	962±494
p_2	2845±876	–	2845±876	–	–	–
D	–	–	–	45 793±24 776	18.7±1095	45 793±24 776
χ^2	131.37	152.04	131.37	142.61	152.06	142.61
σ^2	6.256	6.911	6.256	6.791	6.912	6.791

[a] Number in parentheses depict the number of adjustable parameters of each equation.
[b] r means a reduced equation including only statistically significant parameters.

Excel spreadsheets using the Monte-Carlo algorithm suggested in [21] to solve the fitting problem. As criteria for the performance of each equation we used the value of the sum of squares of residuals (χ^2) and the standard error of the estimate (σ^2) defined as $\sigma^2 = \chi^2/(N-n)$, where N is the number of data points and n is the number of the adjustable parameters. Other criteria, like the numerical behavior and irregularities in the shape of the theoretical response surfaces, are also taken into account and discussed. The standard errors of the fitting parameters were calculated using the two procedures of the curvature matrix method, i.e. the CM1 and CM2 procedures [21]. Note that for the systems of hydroxizine and benzoic acid in methanol–water mobile phases, we have adopted the transformation of the φ values suggested in [23]. Thus instead of φ in the various equations, we used the difference $\varphi - \varphi_r$, where φ_r is a reference value of φ. The values of φ_r used were 0.65 for the first system and 0.3 for the second one.

We should also point out that the plots of ϵ vs. φ and $1/\epsilon$ vs. φ are linear in all cases. This means that $\epsilon_2 = \epsilon_2' = 0$ and therefore $e_2 = r_2 = q_2 = 0$ are valid for the systems we studied. However, in order to have a complete picture about the behavior of Eqs. (16)–(21), we examined (a) the above limiting case including the case where $g_1 = 0$, (b) the general case where $q_2 \neq 0$ and $e_2 \neq 0$, and (c) the case where the fitted equation includes only statistically significant parameters. A parameter is significant from a statistical point of view if the ratio of its absolute value to its uncertainty is greater than unity.

The obtained results for Eqs. (16), (17), (19) and (20) are given in Tables 3–6. Eqs. (18) and (21) are not included in these Tables for the following reason. These two equations exhibit the worst numerical behavior. Thus, we were able to determine the lowest value of χ^2 only after several applications of the Monte-Carlo routine and by changing at every run the ranges of the initial estimates. But even in this case the lowest value of χ^2 does not always correspond to a certain set of values for the adjustable parameters. For example, Table 7 shows two sets of adjustable parameters that correspond to the same value of $\chi^2 = 4.848166$ when Eq. (18) is fitted to the retention data of adenosine in acetonitrile–water buffers. It is seen that they differ only in the values of k_1^0 and pK_r^*. Note that there are numerous such sets of values of the fitted parameters that correspond to $\chi^2 = 4.848166$. For example, if we put $k_1^0 = 1/100\,000$, keep it constant and run the Solver, we obtain $pK_r^* = -6.351$ and $\chi^2 = 4.848166$, whereas if we put $k_1^0 = 100\,000$, we obtain $pK_r^* = 3.649$ with the same value of χ^2. The same or similar behavior has been observed in all applications of Eq. (18) to the different chromatographic systems and in most applications of Eq. (21). It is evident that these two equations cannot be used to gain physical insights. For example, it is impossible to calculate even approximately chromatographic pK_a values from the

Table 4
Fitted parameters for Eqs. (19)–(20) using ${}^{w}_{w}$pH values

Eq.:	19(8)	19(7)	19(r)	20(8)	20(7)	20(r)
Hydroxizine in methanol–water buffers						
k_0^0	17.6±0.6	17.6±0.2	17.7±0.5	17.6±0.6	17.6±0.5	17.7±0.5
k_1^0	7.0±0.5	7.0±0.2	7.1±0.4	7.0±0.5	7.0±0.4	7.1±0.4
pK*	5.35±0.13	5.36±0.05	5.32±0.08	5.35±0.13	5.36±0.11	5.32±0.08
c_0	−135±179	−164±569	−204±146	−135±179	−158±133	−204±146
c_1	−119±167	−148±52	−183±137	−119±169	−142±124	−183±137
b	87.2±78	99.6±23	117±58	87.2±78	96.9±56	117±58
h_1	−0.5±20	3.5±2.4	–	−16.8±95	–	–
h_2	45.5±214	–	–	–	–	–
D	–	–	–	−477±2240	−85.1±138	–
χ^2	1.011	1.021	1.079	1.011	1.018	1.079
σ^2	0.253	0.204	0.180	0.253	0.203	0.180
Benzoic acid in methanol–water buffers						
k_0^0	5.44±0.03	5.44±0.04	5.44±0.04	5.44±0.03	5.44±0.03	5.44±0.03
k_1^0	0.22±0.02	0.22±0.03	0.22±0.03	0.22±0.02	0.22±0.02	0.22±0.02
pK*	4.26±0.01	4.26±0.01	4.26±0.01	4.26±0.01	4.26±0.01	4.26±0.01
c_0	7.1±0.09	7.1±0.1	7.1±0.1	7.1±0.09	7.1±0.08	7.1±0.08
c_1	9.13±2.4	9.1±3.5	9.1±3.5	9.1±2.4	9.1±2.3	9.1±2.3
b	0.16±0.19	0.14±0.2	0.14±0.2	0.16±0.18	0.15±0.2	0.15±0.2
h_1	−2.8±1.3	−2.6±0.7	−2.6±0.7	1.4±18	–	–
h_2	1.4±8	–	–	–	–	–
D	–	–	–	177±817	115±22	115±22
χ^2	0.065	0.065	0.065	0.065	0.065	0.065
σ^2	0.0023	0.0022	0.0022	0.0023	0.0022	0.0022
Adenosine in acetonitrile–water buffers						
k_0^0	1.02±0.32	1.05±0.3	0.96±0.19	1.08±0.32	1.05±0.31	0.96±0.19
k_1^0	22.6±0.2	22.6±0.2	22.6±0.2	22.6±0.2	22.6±0.2	22.6±0.2
pK*	3.48±0.05	3.48±0.05	3.48±0.03	3.48±0.05	3.48±0.04	3.48±0.03
c_0	−1.8±23	5.2±25	–	8.7±27	4.4±24	–
c_1	66.7±1.9	66.9±1.8	67.2±1.5	66.9±1.8	66.8±1.8	67.2±1.5
b	22.0±4	20.8±3.2	21.0±3.1	20.1±3.3	20.7±3.2	21.0±3.1
h_1	3.6±18	−3.4±14	–	85.3±135	–	–
h_2	−403±458	–	–	–	–	–
D	–	–	–	8566±12930	422±1352	–
χ^2	2.216	2.222	2.251	2.176	2.218	2.251
σ^2	0.111	0.106	0.098	0.109	0.106	0.098
Adenosine in methanol–water buffers						
k_0^0	1.01±0.42	1.01±0.41	1.04±0.36	1.01±0.42	1.07±0.40	1.04±0.36
k_1^0	22.6±0.3	22.6±0.3	22.6±0.2	22.6±0.3	22.6±0.3	22.6±0.4
pK*	3.47±0.06	3.47±0.05	3.47±0.05	3.47±0.06	3.47±0.06	3.47±0.05
c_0	−74.9±60	−77.2±52	−40.3±19	−79.0±59	−18.3±25	–
c_1	−18.2±29	−19.4±26	–	−20.3±29	11.7±6	−40.3±19
b	51.1±24	52.0±21	34.9±1.0	52.7±23	21.3±8.3	35.0±1
h_1	0.01±9	−0.7±5	–	−6.0±71	–	–
h_2	−8.6±83	–	–	–	–	–
D	–	–	–	−397±5197	49.3±378	–
χ^2	3.848	3.850	3.926	3.849	3.891	3.926
σ^2	0.167	0.160	0.151	0.167	0.162	0.151

Table 4. Continued

Eq.:	19(8)	19(7)	19(r)	20(8)	20(7)	20(r)
5-hydroxyindole-3-acetic acid in isopropanol–water buffers						
k_0^0	119.7±2.1	119.7±2.0	119.7±1.9	119.8±2.0	119.7±2.0	119.7±1.9
k_1^0	11.2±1.4	11.2±1.4	11.1±1.3	11.2±1.4	11.2±1.4	11.1±1.3
pK*	4.38±0.04	4.39±0.04	4.39±0.04	4.38±0.04	4.39±0.04	4.39±0.04
c_0	110±6	110±6	109±5	110±6	110±6	109±5
c_1	72.2±34	72.2±33	71.3±28	72.2±34	72.6±33	71.3±28
b	53±16	55.3±13	52.4±13	52.5±14	52.2±14	52.5±13
h_1	−3.0±32	−0.9±17	–	104±330	–	–
h_2	75.8±951	–	–	–	–	–
D	–	–	–	5224±16 392	65.0±846	–
χ^2	101.52	101.54	101.56	101.00	101.53	101.56
σ^2	4.614	4.415	4.231	4.591	4.414	4.232

values of pK_r^*. Therefore, Eqs. (18) and (21) exhibit serious drawbacks and they should be avoided for modelling retention data.

The most interesting points that arise from the results presented in Tables 3–6 are the following:

1. The replacement of Eq. (9) by Eq. (8) results in a radical improvement of fitting in most of the cases, irrespective of the pH scale used. For example, we observe that if we fit the experimental data of adenosine in acetonitrile–water buffers to the conventional nine-parameter Eq. (16), the value of χ^2 is 3.50, whereas this value drops to 2.222 when we use the seven-parameter Eq. (19). These results are obtained from the ${}^{w}_{w}$pH scale. If the ${}^{s}_{s}$pH scale is used, then the above values of χ^2 change slightly to 3.52 and 2.25, respectively. Thus despite the fact the Eq. (19) with $h_2 = 0$ has two adjustable parameters fewer than Eq. (16) the fit improvement is considerable. Similarly, for the system of adenosine in methanol–water buffers the conventional nine-parameter Eq. (16) gives $\chi^2 = 7.824$, whereas this value becomes less than half ($\chi^2 = 3.850$) by the use of the seven-parameter Eq. (19) (with $h_2 = 0$). This improvement reflects the better performance of Eq. (8) to fit ln k vs. φ data at constant pH values, as found in [1].
2. The shape of the predicted surfaces and in particular the shape of the predicted ln k vs. φ curves at constant pH values may be used as an additional criterion for the validity of a model. In a recent paper we have shown that a low value of χ^2 is not necessarily associated with a physically coherent retention surface [8]. For this reason before we conclude about the suitability of the equations under consideration, we examined all the plots of ln k vs. φ at constant pH obtained from the Eqs. (16), (17), (19) and (20). We excluded Eqs. (18) and (21) due to their bad numerical behavior and the relatively high number of their adjustable parameters. We observed that Eqs. (19) and (20) predict ln k vs. φ plots that in no case exhibit physically meaningless portions. In contrast, such portions, reflecting overfitting problems, may be found in the curves of ln k vs. φ predicted by Eqs. (16) and (17). Two characteristic cases are shown in Figs. 1 and 2, which depict experimental and calculated ln k vs. φ plots at ${}^{w}_{w}$pH=6.9. The curves have been calculated from Eqs. (16) and Eq. (19), respectively. The plots correspond to the retention of adenosine in acetonitrile–water buffers (Fig. 1) and 5-hydroxyindole-3-acetic acid in isopropanol–water buffers (Fig. 2). We observe that the curves predicted by the conventional Eq. (16) exhibit a totally abnormal shape for both experimental systems. Analogous abnormal shapes are observed in the corresponding 3-D plots predicted by Eq. (16) for the dependence of k upon φ and pH, irrespective of the pH scale used.
3. The pK* values and consequently the pK_a values are almost independent of the fitted equation, even in the cases where overfitting problems yield abnormal retention surfaces. It is also interesting to point out that the pK* (pK_a) values of the analytes in the systems of adenosine in methanol–water and acetonitrile–water buffers and 5-hydroxyindole-3-acetic acid in isopropanol–water

Table 5
Fitted parameters for Eqs. (16), (17) using ${}^{s}_{s}$pH values

Eq.:	16(9)	16(8)	16(r)	17(9)	17(8)	17(r)
Hydroxizine in methanol–water buffers						
k_0^0	17.5±0.5	17.5±0.5	17.4±0.5	17.5±0.5	17.5±0.5	17.4±0.5
k_1^0	7.1±0.5	7.1±0.5	7.0±0.5	7.2±0.5	7.1±0.5	7.0±0.5
pK*	6.36±0.11	6.37±0.11	6.42±0.10	6.36±0.11	6.37±0.10	6.42±0.10
h_1	−16.3±4.4	−14.6±4.4	−10.2±1.2	−32.1±4.5	−11.2±4.5	−10.2±1.2
h_2	25.3±50	7.0±51	–	12.4±51	5.9±51	–
s_0	−8.1±1.9	−8.0±1.9	−8.5±0.5	−8.2±1.9	−7.9±1.9	−8.5±0.5
t_0	−8.2±21	−9.5±21	–	−6.5±21	−10.1±21	–
p_1	−4.8±23	−3.4±6.5	–	−20.3±114	–	–
p_2	16.0±256	–	–	–	–	–
D	–	–	–	−396±2683	77.7±153	–
χ^2	1.328	1.329	1.426	1.329	1.330	1.426
σ^2	0.443	0.332	0.204	0.443	0.332	0.204
Benzoic acid in methanol–water buffers						
k_0^0	5.40±0.03	5.40±0.03	5.40±0.03	5.40±0.03	5.41±0.04	5.41±0.04
k_1^0	0.23±0.03	0.24±0.02	0.24±0.02	0.23±0.02	0.24±0.02	0.24±0.02
pK*	4.67±0.01	4.67±0.01	4.67±0.01	4.67±0.01	4.66±0.01	4.66±0.01
h_1	124±30	126±28	126±28	118±41	134±6	134±6
h_2	−1381±304	−1403±277	−1403±277	−1379±303	−1428±42	−1428±42
s_0	−7.4±0.25	−7.4±0.2	−7.4±0.2	−7.4±0.2	−7.5±0.2	−7.5±0.2
t_0	1.7±1.4	1.9±1.2	1.9±1.2	1.7±1.4	2.1±1.1	2.1±1.1
p_1	−6.0±1.5	−6.4±0.5	−6.4±0.5	−12.3±21	–	–
p_2	−2.4±9	–	–	–	–	–
D	–	–	–	−263±940	283±23	283±23
χ^2	0.071	0.071	0.071	0.071	0.071	0.071
σ^2	0.0026	0.0025	0.0025	0.0026	0.0025	0.0025
Adenosine in acetonitrile–water buffers						
k_0^0	0.93±0.38	0.99±0.49	0.69±0.16	1.08±0.48	0.99±0.48	1.12±0.41
k_1^0	22.6±0.3	22.4±0.3	22.5±0.3	22.5±0.3	22.4±0.3	22.5±0.3
pK*	3.48±0.06	3.48±0.07	3.45±0.04	3.48±0.07	3.47±0.07	3.48±0.06
h_1	−61.1±21	−83.8±23	−59.0±18	204±204	−72.7±2.8	191±194
h_2	−678±376	421±48	−752±355	417±48	419±48	417±47
s_0	−9.9±21	−11.3±53	–	−9.4±52	−7.0±50	−20.9±21
t_0	42.6±220	−53.9±986	–	−269±1047	−126±947	–
p_1	18.7±22	−11.0±24	20.9±19	276±205	–	263±194
p_2	−1385±403	–	−1460±381	–	–	–
D	–	–	–	27 860±19 500	1391±2262	26 439±18 448
χ^2	3.518	4.861	3.647	4.412	4.821	4.428
σ^2	0.185	0.243	0.192	0.232	0.241	0.221
Adenosine in methanol–water buffers						
k_0^0	1.17±0.57	1.21±0.55	0.71±0.25	1.18±0.60	1.22±0.55	0.71±0.25
k_1^0	22.3±0.4	22.4±0.4	22.3±0.4	22.4±0.4	22.36±0.4	22.32±0.4
pK*	3.49±0.08	3.50±0.08	3.46±0.06	3.49±0.08	3.50±.08	3.46±0.06
h_1	−36.6±13	−27.4±8	−24.7±1	−121±101	−24.5±1.1	−24.7±1
h_2	187±115	82.2±10	83.4±9	80.2±10	82.4±10	83.4±9
s_0	2.3±25	−7.6±18	–	1.3±23	−8.4±18	–
t_0	−102±254	13.1±128	–	−92.6±241	17.3±127	–
p_1	−12.4±14	−2.9±8	–	−96.6±101	–	–
p_2	107±118	–	–	–	–	–
D	–	–	–	−6927±7353	186±601	–
χ^2	7.805	8.051	8.761	7.777	8.064	8.761
σ^2	0.355	0.350	0.398	0.353	0.351	0.337

Table 5. Continued

Eq.:	16(9)	16(8)	16(r)	17(9)	17(8)	17(r)
5-hydroxyindole-3-acetic acid in isopropanol–water buffers						
k_0^0	119.7±2.4	119.1±2.5	119.7±2.4	119.2±2.4	119.1±2.5	119.2±2.4
k_1^0	11.0±1.6	11.0±1.7	11.0±1.6	10.8±1.7	11.0±1.7	10.8±1.7
pK*	4.38±0.05	4.39±0.05	4.38±0.05	4.40±0.05	4.39±0.05	4.40±0.05
h_1	−138±61	−93.5±47	−138±61	895±500	−92.0±36	895±500
h_2	4036±1345	961±935	4036±1345	775±696	973±947	775±696
s_0	−141±9	−125±6	−141±9	−130±7	−125±6	−130±7
t_0	2473±390	1447±161	2473±390	1846±301	1447±161	1846±301
p_1	−46.1±38	−2.5±22	−46.1±38	969±506	–	969±506
p_2	2840±878	–	2840±878	–	–	–
D	–	–	–	46 277±25 382	186±1097	46 277±25 382
χ^2	131.75	152.29	131.75	142.63	152.17	142.63
σ^2	6.274	6.922	6.274	6.792	6.917	6.792

buffers are independent of the pH scale. The same is valid for the benzoic acid in methanol–water buffers if we extrapolate the pK* value to $\varphi = 0$. Note that in Tables 3–6 the pK* values of benzoic acid are referred to 30% (v/v) methanol–water buffers. The extrapolation can be easily done if we do not replace φ in the various equations by the difference $\varphi - 0.3$. In this case ${}^{w}_{w}$pH and ${}^{s}_{s}$pH scales give precisely the same result, pK* = 3.94±0.07, averaged over all equations used. However, the corresponding extrapolation for hydroxizine from 65% methanol–water buffers is completely unreliable. In general, it is seen that the results of the present study show that the pH scale does not affect the calculated pK_a values provided that they refer to $\varphi = 0$. In

Table 6
Fitted parameters for Eqs. (19), (20) using ${}^{s}_{s}$pH values

Eq.:	19(8)	19(7)	19(r)	20(8)	20(7)	20(r)
Hydroxizine in methanol–water buffers						
k_0^0	17.5±0.6	17.5±0.6	17.4±0.5	17.5±0.6	17.5±0.6	17.4±0.5
k_1^0	7.2±0.5	7.1±0.5	7.0±0.4	7.2±0.5	7.1±0.5	7.0±0.4
pK*	6.36±0.13	6.37±0.13	6.42±0.10	6.36±0.13	6.37±0.13	6.42±0.10
c_0	−138±128	−152±140	−127±98	−126±118	−155±148	−127±98
c_1	−123±120	−137±132	−114±93	−112±110	−140±139	−114±93
b	88.7±56	94.6±60	82.9±43	83.8±53	96.2±63	82.9±43
h_1	−5.4±7	−3.3±7	–	−23.7±7	–	–
h_2	24.0±214	–	–	–	–	–
D	–	–	–	−477±2241	75±160	–
χ^2	1.331	1.334	1.382	1.333	1.335	1.382
σ^2	0.333	0.267	0.345	0.333	0.267	0.345
Benzoic acid in methanol–water buffers						
k_0^0	5.41±0.03	5.41±0.04	5.40±0.03	5.41±0.03	5.41±0.03	5.41±0.03
k_1^0	0.24±0.02	0.22±0.03	0.24±0.02	0.24±0.02	0.24±0.02	0.24±0.02
pK*	4.66±0.01	4.66±0.01	4.67±0.01	4.66±0.01	4.66±0.01	4.66±0.01
c_0	7.1±0.1	7.1±0.1	7.1±0.1	7.1±0.1	7.1±0.1	7.1±0.1
c_1	8.8±2.3	8.8±2.2	8.8±2.2	8.8±2.3	8.8±2.2	8.8±2.2
b	0.14±0.19	0.14±0.2	–	0.15±0.19	0.17±0.2	0.17±0.2
h_1	−6.1±1.4	−6.1±0.5	−6.0±0.5	−4.4±20	–	–
h_2	4.2±8	–	–	–	–	–
D	–	–	–	75.9±885	270±23	270±23
χ^2	0.073	0.073	0.075	0.073	0.073	0.073
σ^2	0.0026	0.0025	0.0025	0.0026	0.0025	0.0025

Table 6. Continued

Eq.:	19(8)	19(7)	19(r)	20(8)	20(7)	20(r)
Adenosine in acetonitrile–water buffers						
k_0^0	1.03±0.32	1.06±0.3	0.92±0.19	1.09±0.32	1.06±0.31	0.92±0.19
k_1^0	22.6±0.2	22.6±0.2	22.6±0.2	22.6±0.2	22.6±0.2	22.6±0.2
pK*	3.48±0.05	3.48±0.05	3.48±0.04	3.48±0.05	3.48±0.04	3.48±0.03
c_0	−2.2±23	4.8±24	–	8.3±27	4.4±24	–
c_1	66.7±1.9	66.9±1.8	67.5±1.5	66.9±1.8	66.8±1.8	67.5±1.5
b	22.1±4	20.7±3.2	21.1±3.1	20.1±3.3	20.7±3.2	21.1±3.1
h_1	−0.6±18	−8.2±14	–	81.1±137	–	–
h_2	−423±452	–	–	–	–	–
D	–	–	–	8617±13 107	872±1361	–
χ^2	2.237	2.245	2.336	2.200	2.237	2.336
σ^2	0.112	0.107	0.102	0.110	0.107	0.102
Adenosine in methanol–water buffers						
k_0^0	1.01±0.42	1.01±0.41	1.11±0.36	1.01±0.42	1.07±0.40	1.11±0.36
k_1^0	22.6±0.3	22.6±0.3	22.7±0.3	22.6±0.3	22.6±0.3	22.66±0.3
pK*	3.47±0.06	3.47±0.06	3.48±0.05	3.47±0.06	3.47±0.06	3.48±0.05
c_0	−77.7±60	−80.0±52	−30.2±19	−81.8±59	−18.5±24	−30.2±19
c_1	−19.1±29	−20.3±25	–	−21.3±29	11.9±6	–
b	51.8±23	52.8±20	35.2±1.0	53.5±23	21.1±8.1	35.2±1.1
h_1	−2.8±9	−3.5±5	–	−9.2±72	–	–
h_2	−8.9±85	–	–	–	–	–
D	–	–	–	−428±5272	256±383	–
χ^2	3.871	3.873	4.061	3.872	3.916	4.061
σ^2	0.168	0.161	0.156	0.168	0.163	0.156
5-hydroxyindole-3-acetic acid in isopropanol–water buffers						
k_0^0	119.7±2.1	119.7±2.0	119.6±1.9	119.8±2.0	119.7±2.0	119.6±1.9
k_1^0	11.2±1.4	11.2±1.4	11.1±1.3	11.2±1.4	11.2±1.4	11.1±1.3
pK*	4.38±0.04	4.39±0.04	4.39±0.04	4.38±0.04	4.39±0.04	4.39±0.04
c_0	110±6	110±6	109±5	110±6	110±6	109±5
c_1	72.2±34	72.2±33	68.0±28	72.1±34	72.5±33	67.9±28
b	53±16	52.4±13	53.3±13	52.6±14	52.3±13	53.3±13
h_1	−6.1±32	−4.5±17	–	101±331	–	–
h_2	58.3±954	–	–	–	–	–
D	–	–	–	5257±16 446	240±846	–
χ^2	101.55	101.56	101.84	101.02	101.51	101.84
σ^2	4.616	4.416	4.243	4.592	4.414	4.243

contrast, the pK* (pK_a) values calculated in organic modifier–water buffers are strongly affected by the pH scale and reliable values can be obtained only if the ${}^{s}_{s}$pH scale is used.

4. From the pK* values in water buffers the pK_a can be calculated from $K_a = K^*\gamma_0^m/\gamma_1^m$ taking into account that the activity coefficient of the uncharged species can be eliminated whereas that of the ions can be calculated from the Debye–Hückel equation $\log \gamma_i^m = -0.509I/(1+I)$, where I is the ionic strength of the solution. Thus we found the following pK_a values: 4.03 for benzoic acid, 3.39 for adenosine and 4.44 for 5-hydroxyindole-3-acetic acid. If these values are compared to the potentiometric or optical values, 4.2 for benzoic acid [24], 3.52 for adenosine (at $T = 293$ K) [25] and 4.4 for 5-hydroxyindole-3-acetic acid, we observe that the differences are rather small. However, within the frames of the present study we cannot conclude about the origin or the physical meaning of these differences. For example, in the case of the benzoic acid the difference between the two pK_a values 4.03 and 4.2 might be due to the extrapolation used to find the value 4.03 but we cannot verify it. It is evident that there is need of a more careful study

Table 7
Two different sets for the fitted parameters of Eq. (18) that correspond to the same χ^2 value and their standard errors calculated using the CM1 and CM2 procedures. System: adenosine in acetonitrile–water buffers, pH scale: ${}^{w}_{w}$pH

	Parameters		Standard errors			
No.:	1	2	1		2	
			CM1	CM2	CM1	CM2
k_0^0	0.994	0.994	0.505	0.482	0.505	0.483
k_1^0	16.518	23.117	14 245 443.8	241.056	0.505	57.445
pK_r^*	−0.133	0.013	374 539.3	6.338	1029.394	1.079
h_1	−72.912	−72.913	2.849	2.785	2.849	2.784
h_2	421.510	421.515	49.201	44.436	49.201	44.435
s_0	−13.415	−13.422	55.378	52.892	55.378	53.241
t_0	−14.003	−14.017	1029.394	966.026	1029.394	974.874
e_0	0.288	0.288	0.006	0.005	0.006	0.005
e_1	−0.189	−0.189	0.874	0.814	0.874	0.813
e_2	–	–				
χ^2	4.848166	4.848166				

on this issue, which though falls beyond the target of the present investigation that was the extension of our previous theoretical treatment on the retention mechanism to the combined effect of pH and organic modifier content on the retention in RPLC.

6. Conclusions

Taking into account all the above findings we readily conclude that from the equations we studied, Eqs. (19) and (20) work more satisfactorily, irrespective of the pH scale used; they exhibit good numerical behavior, they do not predict physically meaningless retention surfaces and they are the most accurate since they give the lowest values of χ^2 and σ^2. Their performance is equally good when we adopt $h_2 = 0$ for Eq. (19) and $h_1 = 0$ for Eq. (20), i.e. when they work as seven-parameter equations. In contrast, the conventional Eq. (16) is less accurate, because it may predict retention curves of ln k vs. φ

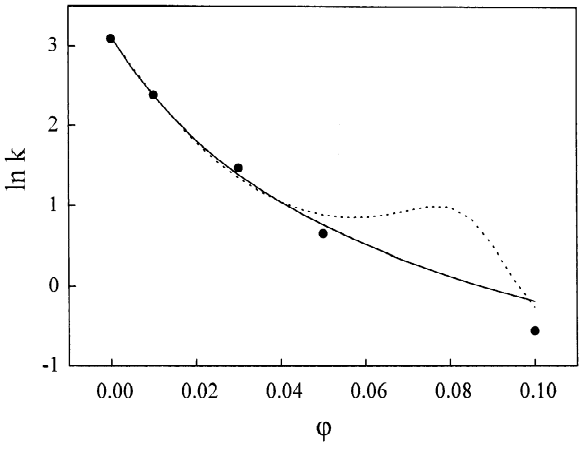

Fig. 1. Retention plots of adenosine in acetonitrile–water mobile phase at ${}^{w}_{w}$pH = 6.9. Points are experimental data from [19], curves have been calculated from the complete Eq. (16) (- - -) and Eq. (19) (—) using the relevant parameters of Tables 1 and 2.

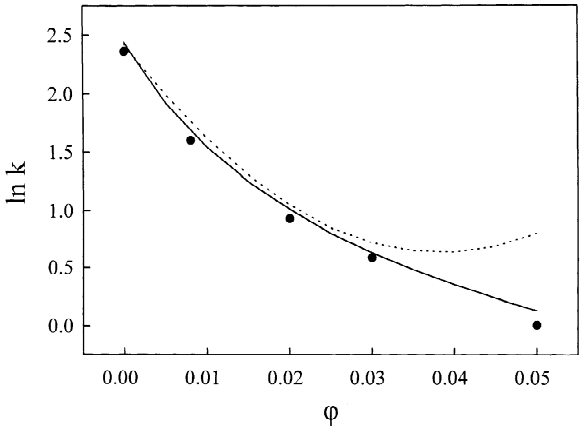

Fig. 2. Retention plots of 5-hydroxyindole-3-acetic acid in isopropanol–water buffers at ${}^{w}_{w}$pH = 6.9. Points are experimental data from [23], curves have been calculated from the complete Eq. (16) (- - -) and Eq. (19) (—) using the relevant parameters of Tables 1 and 2.

with physically meaningless portions. Of the two equations with the good performance, Eq. (19) is the most flexible, since there is no need of measurements of the dielectric constant ϵ.

Although Eq. (19) should be preferred for modelling of retention data and further for separation optimization, the chromatographically calculated pK_a values are almost independent of the fitted equation, even in the cases where overfitting problems yield abnormal retention surfaces. In addition, the pH scale seems not to affect the calculated pK_a values when they are referred to aqueous buffers. In contrast, the pK_a values calculated in organic modifier–water buffers are strongly affected by the pH scale and reliable values can be obtained only if the $^{s}_{s}$pH scale is used.

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