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Simple models for the effect of aliphatic alcohol additives on the retention in reversed-phase liquid chromatography

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ABSTRACT

Four retention models for the effect of aliphatic alcohol additives on the retention of analytes in reversed-phase liquid chromatography have been developed following either a semi-thermodynamic treatment or an empirical approach. Their performance was tested using the experimental retention times of six non-polar analytes (alkylbenzenes) and ten o-phthalaldehyde derivatives of amino acids under different isocratic chromatographic runs when a small amount of ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol or 1-heptanol was added to methanol/water mixtures containing a constant amount of methanol. It was shown that for the structurally simple alkylbenzenes all the models can be adopted for retention prediction with good results. In contrast, just one out of four models, that with the fewest approximations, predicts satisfactorily the retention properties of amino acids derivatives. However, the most interesting feature is that this model can predict the effect of an alcohol-additive on the retention properties of solutes, even if this additive was not used in chromatographic runs done for the fitting procedure, provided that it belongs to the same homologous series of alkanols. This feature is also observed in all models described the retention of alkylbenzenes.

1. Introduction

The composition of the mobile phase in liquid chromatography plays an important role both in isocratic and gradient elution. The use of binary solvent mixtures provides the most common solution to the problem of separation optimization but difficult separations can often be resolved when either ternary isocratic [1–4] or ternary gradient chromatography [5–9] is employed. However, the commonly used organic modifiers in reversed-phase liquid chromatography RPLC systems are methanol (MeOH) and acetonitrile (MeCN) and only few publications, to our knowledge, focused on enhancing the selectivity using some relatively uncommon organic modifiers [10–15]. In most of these studies, straight-chain upper alkanols at low concentration were used either as organic modifiers in binary solvent mixtures [10,11] or as additives together with MeOH or MeCN in ternary mobile phases [13–15] to modify and improve the separations. However, no systematic study for the combined effect of the type and the concentration of the additive on solute retention has been reported in literature.

The present study was focused for the first time on developing and testing simple models correlating retention to the number of alkyl chain of alkanol additives (short and medium chain-length alcohols) as well as to their content in MeOH/water mixtures with a constant concentration of MeOH. Moreover, it is the aim of this paper to explore the possibility of using these models for solute retention prediction in ternary mobile phases consisting of MeOH, water plus any type and concentration of alkanol additive. For this purpose it was investigated the retention of two mixtures of solutes under different isocratic chromatographic runs using a variety of ternary mixtures of MeOH, alcohol additive and water as mobile phases. One mixture consisted of six structurally simple non-polar solutes (alkylbenzenes) and the other of ten o-phthalaldehyde (OPA) derivatives of amino acids, i.e. solutes with polar or/and ionizable groups. For these ternary systems, a small concentration of various alcohols including MeOH, ethanol (EtOH), 1-propanol (PrOH), 1-butanol (BuOH), 1-pentanol (PeOH), 1-hexanol (HexOH) or 1-heptanol (HepOH) was added to MeOH/water mixtures containing a constant amount of MeOH.

2. Theory

Retention in RPLC is a very complex process involving a great variety of interactions that are difficult to describe exactly. The complexity of these interactions results in a rather obscure picture about the retention mechanism. The solvophobic theory was one of the first attempts to describe chromatographic retention by means of classical thermodynamics [16–19]. This theory has been criticised by Dill [20], who developed the partition model,

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i.e. a retention model that considers the partitioning of the analyte molecules from the mobile phase into the stationary phase [21–27]. An alternative approach proposed to explain the retention process is the adsorption model, according to which the analyte molecules are adsorbed at a surface solution formed on the tips of the hydrocarbon chains of the stationary phase [20,21,28–40]. The surface solution has the same constituents with those of the mobile phase but with different concentrations. In the majority of the studies, the adsorption model assumes a displacement process; analyte molecules and molecules of the organic modifier co-adsorbed at the interface displace solvent molecules. A combination of adsorption and partition models has been also proposed [34,41–43].

In the present study we adopt the semi-thermodynamic approach developed in [39] in order to derive retention models that will take into account the effect of alkanol additives. For comparison purposes, retention models based on the empirical approach described in [9,40,43–45] are also developed and discussed.

2.1. A semi-thermodynamic approach

Consider that the mobile phase of a chromatographic column consists of the solvent (water), W, the organic modifier, B, the additive, D, and the analyte, A. The additive is an alcohol with n carbon atoms, its concentration is small in comparison to that of the organic modifier, whereas the concentration of the analyte tends to zero. For the retention factor we may write

$$\ln\ k = \ln\ \Phi + \ln\ \frac{\varphi_A^s}{\varphi_A^m} \tag{1}$$

where Φ is the phase ratio, and φ_A^s and $\varphi_A^{\bar{m}}$ are the volume fractions of A in the stationary (s) and in the mobile phase (m). To a first approximation the ratio φ_A^s/φ_A^m depends on the mutual interactions of A in the mobile and stationary phase. However, at a certain column and when the concentration of the organic modifier, B, is kept constant, the interactions of A with W and B, A–W and A–B interactions, are expected to have a constant contribution to $\ln k$, whereas the contribution from the A–D interactions will depend on both the additive concentration in the mobile phase, φ_D, and the number of carbon atoms, n, of the additive.

A simple but phenomenological approach to treat the A–D interactions is to assume the existence of the following equilibrium in the mobile phase [39]

$$\mathrm{A} + \mathrm{D} \Leftrightarrow \mathrm{AD} \equiv \mathrm{P} \tag{2}$$

In this approach a measure of the A–D interactions is the equilibrium constant K

$$K = \frac{\varphi_P^m}{\varphi_A^m \varphi_D^m} \tag{3}$$

where φ_D^m and φ_P^m are the volume fractions of D and the associate P in the mobile phase. It is evident that strong attractive interactions between A and D shift the equilibrium of Eq. (2) to the right increasing K. Note that due to Eq. (2) the analyte A in the mobile phase exists in the form of monomers (A) and the associate (AD ≡ P). Thus the denominator in the fraction of Eq. (1), φ_A^m, should be replaced by the total volume fraction of the analyte, $\varphi_{A,total}^m = \varphi_A^m + \varphi_P^m$. Due to the equilibrium expressed by Eq. (2), Eq. (1) yields

$$\ln\ k = \ln\ \Phi + \ln\ \frac{\varphi_A^s}{\varphi_{A,total}^m} = \ln\ \Phi + \ln\ \frac{\varphi_A^s}{\varphi_A^m + \varphi_P^m} \tag{4}$$

Eq. (4) in combination with Eq. (3) results in

$$\ln\ k = \ln\ \Phi + \ln\ \frac{\varphi_A^s}{\varphi_A^m(1 + K\varphi_D^m)} \tag{5}$$

Note that

$$K = e^{-\Delta G/RT} \tag{6}$$

where the free energy ΔG depends on the A–D interactions and due to its additive property we may write

$$\Delta G = \Delta G_{\mathrm{OH}} + n\Delta G_{\mathrm{C}} \tag{7}$$

Here, the term ΔG_{OH} is due to the interaction of A with the hydroxyl group of the alcohol and the term ΔG_{C} expresses the contribution of the interactions of A with each of the carbon groups of the alcohol. Therefore K way be written as

$$K = e^{-\Delta G_{\mathrm{OH}}/RT} e^{-n\Delta G_{\mathrm{C}}/RT} = K_{\mathrm{OH}} e^{-n\Delta G_{\mathrm{C}}/RT} \tag{8}$$

Now to proceed further and determine an analytical expression for $\ln k$ we need to make assumptions on the retention mechanism. For simplicity, we adopt that retention is due to the following simple adsorption process

$$\mathrm{A}^m + \mathrm{S}^s \Leftrightarrow \mathrm{A}^s + \mathrm{S}^m, \quad \mathrm{D}^m + \mathrm{S}^s \Leftrightarrow \mathrm{D}^s + \mathrm{S}^m \tag{9}$$

Here, S denotes the mixture of constant composition of the water and the organic modifier. Therefore, assuming a Langmuirian process, the equilibrium may be expressed as

$$\frac{\varphi_A^s}{1 - \varphi_A^s - \varphi_D^s} = \beta_A \frac{\varphi_A^m}{1 - \varphi_D} \approx \beta_A \varphi_A^m \tag{10}$$

$$\frac{\varphi_D^s}{1 - \varphi_A^s - \varphi_D^s} = \beta_D \frac{\varphi_D}{1 - \varphi_D} \approx \beta_D \varphi_D \tag{11}$$

where for simplicity superscript m in φ_D is omitted. Note that (a) $1 - \varphi_D \approx 1$ because the concentration of the additive in the mobile phase is small and (b) in this simplified approach the mixture of the water and the organic modifier is treated as a single compound, S, because it has a constant composition both in the mobile and the stationary phase. That is, we assume that the additive does not alter significantly the ratio modifier/water either in the mobile or in the stationary phase. From the above equations we obtain

$$\varphi_A^s = \frac{\beta_A \varphi_A^m}{1 + \beta_A \varphi_A^m + \beta_D \varphi_D} \Rightarrow \varphi_A^s = \frac{\beta_A \varphi_A^m}{1 + \beta_D \varphi_D} \tag{12}$$

since the concentration of the analyte, φ_A^m, tends to zero. Eq. (12) in combination with Eq. (5) yields

$$\ln\ k = \ln\ \Phi + \ln\ \frac{1}{1 + K\varphi_D} + \ln\ \frac{\beta_A}{1 + \beta_D \varphi_D} \tag{13}$$

and therefore

$$\frac{1}{k} = c_0 + c_1 \varphi_D + c_2 \varphi_D^2 \tag{14}$$

where

$$c_0 = \frac{1}{\Phi \beta_A}, \quad c_1 = \frac{\beta_D + K}{\Phi \beta_A}, \quad c_2 = \frac{\beta_D K}{\Phi \beta_A} \tag{15}$$

Alternatively, Eq. (14) is written as

$$\frac{1}{k} = c_0 + c_0(\beta_D + K)\varphi_D + c_0 \beta_D K \varphi_D^2 \tag{16}$$

In this equation parameter β_D may be written by an expression similar to that of Eq. (8), because β_D is related to the free energy of adsorption of the additive alcohol ($\Delta G_{ads} = -RT \ln \beta_D$). Therefore, we may write $\Delta G_{ads} = \Delta G_{a,\mathrm{OH}} + n\,\Delta G_{a,\mathrm{C}}$, where the term $\Delta G_{a,\mathrm{OH}}$ is due to the interactions of the hydroxyl group of the alcohol with the adsorbing surface and the term $\Delta G_{a,\mathrm{C}}$ expresses the contribution of the adsorption interactions of each of the carbon groups of the alcohol. Thus we have

$$\beta_D = \beta_{\mathrm{OH}} e^{-n\Delta G_{a,\mathrm{C}}/RT} \tag{17}$$

Table 1
Experimental retention times (in min) of alkylbenzenes obtained in the Nucleosil column.

No.	n	φ_D	B	T	EB	*i*PB	PB	*t*BB
1	2	0.05	4.69	6.87	9.83	13.58	15.45	18.28
2	2	0.1	3.85	5.20	6.85	8.82	9.83	11.20
3	4	0.05	4.41	6.27	8.70	11.67	13.15	15.30
4	6	0.05	4.35	6.04	8.14	10.62	11.86	13.54
5	6	0.1	3.42	4.28	5.22	6.21	6.71	7.29
6	2	0.025	5.35	8.22	12.31	17.68	20.34	24.49
7	2	0.075	4.24	5.98	8.23	11.00	12.39	14.39
8	4	0.025	4.95	7.39	10.77	15.10	17.24	20.54
9	4	0.075	3.89	5.23	6.88	8.80	9.76	11.08
10	4	0.1	3.55	4.60	5.82	7.19	7.89	8.79
11	6	0.025	4.88	7.28	10.51	14.57	16.59	19.57
12	6	0.075	3.81	4.99	6.38	7.90	8.67	9.63
13	1	0.05	4.85	7.20	10.42	14.61	16.72	19.92
14	3	0.05	4.59	6.62	9.30	12.67	14.33	16.84
15	5	0.05	4.39	6.18	8.49	11.26	12.64	14.56
16	7	0.05	4.33	5.96	7.95	10.19	11.32	12.77
17	1	0.1	4.04	5.58	7.51	9.83	11.02	12.68
18	3	0.1	3.66	4.84	6.25	7.88	8.72	9.81
19	5	0.1	3.47	4.43	5.51	6.68	7.27	7.98
20	7	0.1	3.39	4.21	5.06	5.90	6.34	6.81

Note that β_D is a property of the additive and therefore β_D as well as β_{OH} and $\Delta G_{a,C}$ are independent of the analyte. Thus, in order to determine β_{OH} and $\Delta G_{a,C}$ we should apply Eq. (16) to the retention data of a group of analytes in the same modifier using a non-linear fitting procedure.

If Eq. (16) is applied to the retention data of a single analyte, then due to the symmetry of K and β_D in this equation, the fitting always yields $\beta_D = K$. Therefore, for the application of Eq. (16) per analyte we may write $\beta_D = K = \beta$ and therefore

$$\frac{1}{k} = c_0 + c_0 2\beta\varphi_D + c_0\beta^2\varphi_D^2 \tag{18}$$

Moreover, from the expressions of K and β_D, Eqs. (8) and (17), it arises that β may be expressed as

$$\beta = c_1 e^{-nc_2} \tag{19}$$

Thus Eq. (18) is in fact a three-parameter model.

2.2. Empirical models

In Refs. [9,40,43–45] we have shown that when the retention is governed by two variables, say x_1 and x_2, like φ and T or n and φ_D, $\ln k$ may be written as

$$\ln\ k = \ln\ k(x_1)\ \ln\ k(x_2) \tag{20}$$

For small variations in x_1 and x_2, $\ln k(x_1)$ and $\ln k(x_2)$ vary linearly with x_1, x_2 and if $x_1 = n$ and $x_2 = \varphi_D$, then Eq. (20) results in

$$\ln\ k = (a_1 + na_2)(a_3 + \phi_D a_4) \tag{21}$$

which yields

$$\ln\ k = c_0 + c_1\varphi_D + c_2 n + c_3 n\varphi_D \tag{22}$$

Thus Eq. (20) is in fact a way of writing first order general linear models with interaction terms.

An alternative empirical expression for $\ln k$ arises as follows. For a certain additive we may write

$$\ln\ k = \ln\ k_0 - b\varphi_D \tag{23}$$

since its concentration φ_D varies in a limited range. When we use a homologous series of alkanols as additives, b is proved experimentally to vary linearly with n, the number of carbon atoms of alcohols. Therefore, we have the three-parameter expression

$$\ln\ k = c_0 - (c_1 + nc_2)\varphi_D \tag{24}$$

3. Experimental

The liquid chromatography system consisted of a Shimadzu LC-20AD pump, a model 7125 syringe loading sample injector fitted with a 20 μL loop, a Nucleosil column (5 μm, 150 mm × 4.6 mm), an Agilent Zorbax SB-C18 (3.5 μm, 150 mm × 4.6 mm) or an Agilent Zorbax Eclipse-AAA column (3.5 μm, 150 mm × 4.6 mm) thermostatted at 30 °C by a CTO-10AS Shimadzu column oven and a Shimadzu UV-Visible spectrophotometric detector (Model SPD-10A) working at 254 or at 338 nm.

Two different mixtures of solutes were used as model compounds in this paper. The first one consisted of six non-polar solutes: benzene (B), toluene (T), ethylbenzene (EB), isopropylbenzene (*i*PB), propylbenzene (PB) and *tert*-butylbenzene (*t*BB). Alkylbenzene sample solutions were prepared in neat MeOH at a concentration of 5 mM of each compound. In order to investigate the effects brought on retention of these solutes by different alcohol additives in the methanol/water mobile phases, different isocratic chromatographic runs were performed using ternary mixtures of MeOH-alcohol additive-water with a constant concentration of MeOH ($\varphi_{MeOH} = 0.6$). Among the alcohols used as additives in this study (i.e. MeOH, EtOH, PrOH, BuOH, PeOH, HexOH and HepOH) EtOH, BuOH and HexOH were selected for a more systematic study, i.e. the solute retention was studied for five different compositions of these alcohol additives in the mobile phase ranged between $\varphi_D = 0.025$ and $\varphi_D = 0.1$ with 0.025 increments in φ_D values. In contrast, the other alkanols (MeOH, PrOH, PeOH and HepOH) were used only at volume fractions $\varphi_D = 0.05$ and $\varphi_D = 0.1$ in the MeOH/water mobile phase. The experimental retention data recorded by the UV detector at 254 nm under the above mobile phase conditions using alternatively the Nucleosil and the Zorbax SB-C18 column are shown in Tables 1 and 2, respectively.

The second mixture of solutes consisted of ten OPA derivatives of aminoacids: L-Arginine (Arg), Taurine (Tau), beta-(3,4-dihydroxyphenyl)-L-Alanine (Dopa), L-Alanine (Ala), L-Methionine (Met), L-tryptophan (Trp), L-phenylanine (Phe), L-Valine (Val), L-Isoleucine (Ile) and L-Leucine (Leu). The derivatives formed by the reaction of OPA with amino acids in the presence of 2-mercaptoethanol (2-ME), according to the previously published non-automated, manual pre-column derivatization procedure [46] with minor modifications. The detection of derivatized amino acids was performed by the UV detector at 338 nm. Appropriate working concentrations of underivatized amino acids were used in the derivatization procedure by OPA/2-ME reagent (Arg = 5 μg/mL;

Table 2
Experimental retention times in min obtained in the Zorbax SB-C18 column.

No.	n	φ_D	B	T	EB	iPB	PB	tBB
1	2	0.05	4.32	6.63	10.12	14.99	17.02	21.44
2	2	0.1	3.48	4.88	6.83	9.35	10.40	12.54
3	4	0.05	4.01	5.93	8.71	12.41	13.95	17.14
4	6	0.05	3.82	5.45	7.71	10.52	11.72	13.97
5	6	0.1	2.97	3.78	4.78	5.89	6.36	7.16
6	2	0.025	4.84	7.79	12.42	19.10	21.92	28.17
7	2	0.075	3.86	5.66	8.28	11.77	13.24	16.31
8	4	0.025	4.60	7.23	11.26	16.93	19.31	24.48
9	4	0.075	3.51	4.89	6.80	9.19	10.19	12.16
10	4	0.1	3.18	4.23	5.61	7.27	7.97	9.29
11	6	0.025	4.45	6.86	10.43	15.27	17.33	21.51
12	6	0.075	3.32	4.46	5.92	7.63	8.37	9.65
13	1	0.05	4.44	6.94	10.78	16.18	18.45	23.41
14	3	0.05	4.12	6.18	9.24	13.41	15.15	18.85
15	5	0.05	3.93	5.71	8.26	11.55	12.94	15.68
16	7	0.05	3.70	5.19	7.16	9.53	10.56	12.36
17	1	0.1	3.63	5.21	7.45	10.43	11.68	14.27
18	3	0.1	3.28	4.47	6.06	8.06	8.88	10.51
19	5	0.1	3.08	4.02	5.21	6.58	7.15	8.18
20	7	0.1	2.92	3.66	4.51	5.43	5.82	6.44

Tau, Ala = 7.5 μg/mL; Dopa, Met, Trp, Phe, Val = 10 μg/mL; Ile, Leu = 15 μg/mL) so that the peak heights of the OPA-derivatives recorded by UV detector do not differ significantly. The mobile phases were aqueous phosphate buffers (with a total ionic strength of 0.01 M and a pH 2.5) modified with constant concentration of MeOH ($\varphi_{MeOH} = 0.5$) and with different low concentrations of alkanol additives varying between $\varphi_D = 0.01$ and $\varphi_D = 0.07$. The Zorbax Eclipse-AAA column was used for the separations of derivatized amino acids. The experimental retention data obtained under the above chromatographic conditions are shown in Table 3.

The flow rate was 1.0 mL/min and the hold-up time was estimated to be $t_0 = 1.53$, 1.39 and 1.47 min for the Nucleosil, Zorbax SB-C18 and Zorbax Eclipse-AAA column, respectively. All solutions were prepared in volumetric flasks to account for volume contraction.

4. Data analysis

The analysis of data has been performed using Microsoft Excel 2007 spreadsheets, where Solver was used in all fitting procedures. The cost functions adopted were

$$\mathrm{CF} = \sum_{j=1}^{N} (\ln\ k_{j,\mathrm{exp}} - \ln\ k_{j,\mathrm{calc}})^2 \tag{25}$$

for Eqs. (22), (24), and

$$\mathrm{CF} = \sum_{j=1}^{N} w_j \left(\frac{1}{k_{j,\mathrm{exp}}} - \frac{1}{k_{j,\mathrm{calc}}} \right)^2 \tag{26}$$

for Eqs. (16) and (18). In these functions $k_{j,\mathrm{exp}}$, $k_{j,\mathrm{calc}}$ are the experimental and calculated retention factors and w_j is a weighting factor. Using $w_j = k_j^2$, Eq. (26) becomes equivalent to Eq. (25). Note that if we use $w_j = 1$, the fitting is deceptive; it gives very small CF values because $1/k$ is much smaller than $\ln k$, but these low CF values do not correspond to a good prediction in the retention times [39].

The reason that Eq. (26) becomes equivalent to Eq. (25) when $w_j = k_j^2$ is straightforward. In Eq. (25) we minimize a sum of terms of the general form $(\delta \ln k)^2$, whereas in Eq. (26) the terms are of the form $(w\delta(1/k))^2$. When these quantities are quite small, we have

$$\frac{\delta\ \ln\ k}{w\delta(1/k)} = \frac{\delta k/k}{-w\delta k/k^2} = -\frac{k}{w} \Rightarrow (\delta\ \ln\ k)^2 = \frac{k^2}{w^2}(w\delta(1/k))^2 \tag{27}$$

which shows that the use of $w_j = k_j^2$ in Eq. (26) makes this sum equivalent to that of Eq. (25).

In order to fit Eq. (16) we used all the analytes together. The adjustable parameters are: β_{OH}, $\Delta G_{a,C}$, c_0, K_{OH}, and ΔG_C. The last two parameters arise from constant K through Eq. (8). From these parameters, β_{OH} and $\Delta G_{a,C}$ are independent of the analyte and therefore they are forced through the fitting procedure to take a common value for all analytes. On the contrary, parameters c_0, K_{OH},

Table 3
Experimental retention times (in min) of amino acids obtained in the Zorbax Eclipse-AAA column.

No.	n	φ_D	Arg	Tau	Dopa	Ala	Met	Trp	Phe	Val	Ile	Leu
1	2	0.01	2.35	2.98	4.38	7.29	16.90	18.87	25.99	25.99	49.38	49.83
2	2	0.05	2.03	2.45	3.15	5.14	9.80	10.37	14.34	15.07	26.33	26.71
3	2	0.07	1.91	2.23	2.69	4.20	7.31	7.54	10.16	10.87	18.00	18.10
4	6	0.03	1.65	1.79	1.98	2.57	3.85	3.88	4.99	5.45	8.22	8.01
5	6	0.07	1.57	1.59	1.67	2.04	2.49	2.52	2.89	3.10	3.86	3.84
6	1	0.05	2.10	2.55	3.40	5.56	11.21	12.07	16.47	17.02	30.58	30.97
7	2	0.03	2.11	2.60	3.45	5.73	11.44	12.36	17.09	17.80	31.87	32.47
8	3	0.05	1.84	2.15	2.57	4.00	6.89	7.14	9.69	10.35	17.15	17.40
9	4	0.01	2.18	2.70	3.78	6.22	13.19	14.80	20.43	21.02	38.93	39.15
10	4	0.03	1.90	2.25	2.71	4.41	7.98	8.23	11.58	12.30	21.22	21.05
11	4	0.05	1.76	1.89	2.20	3.42	5.36	5.42	7.32	8.07	12.60	12.73
12	4	0.07	1.67	1.78	1.90	2.65	3.94	3.92	5.06	5.50	8.07	8.14
13	5	0.05	1.64	1.77	1.95	2.66	3.73	3.73	4.79	5.19	7.86	7.75
14	6	0.01	1.91	2.24	2.82	4.11	7.85	8.42	11.48	12.37	21.61	21.70
15	6	0.05	1.57	1.66	1.85	2.26	3.01	3.12	3.71	4.01	5.51	5.37

Table 4
Adjustable parameters of the fitting equations to alkylbenzenes data in the Nucleosil column.

	B	T	EB	*i*PB	PB	*t*BB
			Eqs. (16) and (17)			
			Column Nucleosil, $\beta_{OH} = 5.86 \pm 85$, $\Delta G_{a,C}/RT = -0.098 \pm 1.2$			
c_0	0.33 ± 0.36	0.18 ± 0.13	0.106 ± 0.03	0.066 ± 0.04	0.055 ± 0.04	0.042 ± 0.05
K_{OH}	1.55 ± 52	2.861 ± 60	4.69 ± 75	6.86 ± 96	7.54 ± 103	9.2 ± 122
$\Delta G_C/RT$	−0.03 ± 2.9	−0.09 ± 2	−0.11 ± 1.4	−0.126 ± 1.2	−0.13 ± 1.1	−0.13 ± 1.0
			Eqs. (18) and (19)			
c_0	0.342 ± 0.02	0.184 ± 0.02	0.107 ± 0.02	0.066 ± 0.02	0.055 ± 0.02	0.043 ± 0.02
c_1	3.308 ± 0.62	4.194 ± 1.1	5.259 ± 1.9	6.328 ± 3.1	6.625 ± 3.8	7.296 ± 4.8
c_2	−0.081 ± 0.02	−0.094 ± 0.03	−0.102 ± 0.03	−0.11 ± 0.04	−0.113 ± 0.05	−0.117 ± 0.05
			Eq. (22)			
c_0	1.02 ± 0.1	1.63 ± 0.1	2.16 ± 0.09	2.61 ± 0.09	2.77 ± 0.09	2.99 ± 0.08
c_1	−4.97 ± 1.3	−6.07 ± 1.2	−7.31 ± 1.2	−8.24 ± 1.1	−8.42 ± 1.1	−8.94 ± 1.1
c_2	−0.005 ± 0.02	−0.013 ± 0.02	−0.023 ± 0.02	−0.030 ± 0.02	−0.032 ± 0.02	−0.034 ± 0.02
c_3	−0.48 ± 0.29	−0.60 ± 0.28	−0.69 ± 0.27	−0.81 ± 0.25	−0.86 ± 0.26	−0.93 ± 0.24
			Eq. (24)			
c_0	1.001 ± 0.03	1.578 ± 0.03	2.069 ± 0.04	2.486 ± 0.04	2.642 ± 0.05	2.849 ± 0.05
c_1	−4.74 ± 0.47	−5.46 ± 0.51	−6.22 ± 0.62	−6.79 ± 0.72	−6.91 ± 0.75	−7.17 ± 0.82
c_2	−0.534 ± 0.07	−0.753 ± 0.07	−0.958 ± 0.09	−1.169 ± 0.1	−1.239 ± 0.1	−1.369 ± 0.1

and ΔG_C take values depending on the analyte. In what concerns Eq. (18), it was fitted separately to the experimental data of each analyte using as adjustable parameters the constants c_0, c_1, and c_2, through Eq. (19).

5. Results and discussion

In order to evaluate both the fitting and the prediction performance of the models proposed in this paper, the original data depicted in Tables 1–3 were divided into two groups. In particular, the first 5 rows of data in all Tables 1–3 used for fitting and the rest for prediction. The adjustable parameters of Eqs. (16), (18), (22) and (24) obtained from the fittings are shown in Tables 4–6, whereas Table 7 presents the absolute average percent error between experimental and calculated retention times for the fitting and the prediction data as well as the maximum percentage error between experimental and calculated retention times.

In what concerns the standard deviations in Tables 4–6 we should make the following comment. In our study we have calculated the standard error of all parameters using the curvature matrix and the Monte Carlo simulation method we described in Ref. [47]. Both methods gave converged results and only the results of the curvature method are shown in these tables. The reason we used two methods was because we faced the following peculiar case, especially in what concerns the model of Eq. (18). We observe in Table 6 that all the fitting parameters of this equation for Ala, Met, Trp, Phe, Val, Ile and Leu are statistically non significant ($p > 0.05$), because $|t| = c_i/s_{ci} < 2$, where s_{ci} is the standard deviation of c_i. For these analytes the less significant parameter is c_1 and the next non significant parameter is c_0. However, if we remove c_1, Eq. (18) reduces to $1/k = c_0$, whereas if we remove c_0, Eq. (18) reduces to $1/k = 0$, whereas t_R as well as $1/k$ shows a strong dependence upon n and φ_D. A similar behavior is also detected when we apply Eq. (18) to alkylbenzenes (Tables 4 and 5). However, this reduction of Eq. (18) shows that both c_0 and c_1 are always statistically significant. Thus for Eq. (18) the criteria for a fitted parameter to be statistically significant fail to give correct results. We assume that the reason is the fact that parameter c_0 interferes in all terms of Eq. (18) and similarly c_1 is present in the last two terms of Eq. (18).

From Table 7 we observe that all the derived retention models give reasonably good results only in the case of alkylbenzenes. The fitting error is about 1%, although Eq. (22) reduces it to less than 0.3%. The prediction error ranges from 1.6 to 2.8%, where again Eq. (22) presents the smallest error, 1.6%. Finally, the maximum prediction error lies between 6.2% and 11.5%. Again Eq. (22) shows the smallest error. Therefore, Eq. (22) exhibits a slightly better performance; however, the problem is that this equation contains four adjustable parameters.

The overall good performance of the models described above for the case of alkylbenzenes deteriorates significantly when they are applied to amino acids. Table 7 shows that only Eq. (16) gives reasonably good results; the average fitting error is about 2.5% with

Table 5
Adjustable parameters of the fitting equations to alkylbenzenes data in the Zorbax SB-C18 column.

	B	T	EB	*i*PB	PB	*t*BB
			Eqs. (16) and (17)			
			Column Zorbax SB-C18, $\beta_{OH} = 7.35 \pm 32$, $\Delta G_{a,C}/RT = -0.127 \pm 0.37$			
c_0	0.306 ± 0.17	0.159 ± 0.07	0.088 ± 0.03	0.051 ± 0.01	0.043 ± 0.01	0.031 ± 0.01
K_{OH}	1.2 ± 16	2.3 ± 17	3.8 ± 21	5.7 ± 27	6.3 ± 29	7.8 ± 35
$\Delta G_C/RT$	0.006 ± 1.2	−0.098 ± 0.79	−0.127 ± 0.56	−0.146 ± 0.45	−0.149 ± 0.43	−0.159 ± 0.39
			Eqs. (18) and (19)			
c_0	0.329 ± 0.016	0.166 ± 0.016	0.089 ± 0.016	0.051 ± 0.014	0.043 ± 0.014	0.031 ± 0.012
c_1	3.40 ± 0.43	4.32 ± 0.87	5.36 ± 1.6	6.46 ± 2.6	6.80 ± 3.1	7.54 ± 3.9
c_2	−0.105 ± 0.01	−0.117 ± 0.02	−0.127 ± 0.027	−0.136 ± 0.034	−0.138 ± 0.04	−0.144 ± 0.04
			Eq. (22)			
c_0	1.121 ± 0.04	1.785 ± 0.05	2.389 ± 0.04	2.921 ± 0.03	3.081 ± 0.03	3.388 ± 0.02
c_1	−5.74 ± 0.58	−6.74 ± 0.61	−7.88 ± 0.55	−8.90 ± 0.4	−9.14 ± 0.42	−9.77 ± 0.29
c_2	−0.024 ± 0.01	−0.032 ± 0.01	−0.044 ± 0.01	−0.056 ± 0.007	−0.059 ± 0.008	−0.069 ± 0.005
c_3	−0.45 ± 0.13	−0.62 ± 0.14	−0.74 ± 0.12	−0.862 ± 0.09	−0.898 ± 0.09	−0.961 ± 0.07
			Eq. (24)			
c_0	1.023 ± 0.03	1.656 ± 0.04	2.213 ± 0.06	2.695 ± 0.07	2.846 ± 0.07	3.113 ± 0.08
c_1	−4.57 ± 0.53	−5.19 ± 0.68	−5.77 ± 0.89	−6.19 ± 1.1	−6.32 ± 1.2	−6.48 ± 1.4
c_2	−0.746 ± 0.07	−1.011 ± 0.09	−1.27 ± 0.13	−1.54 ± 0.16	−1.60 ± 0.17	−1.78 ± 0.19

Table 6
Adjustable parameters of the fitting equations to amino acids data.

	Arg	Tau	Dopa	Ala	Met	Try	Phe	Val	Ile	Leu
				Eqs. (16) and (17)						
				Column Zorbax Eclipse-AAA, β_{OH} = 6.19 ± 5.2, $\Delta G_{a,C}/RT$ = −0.57 ± 0.13						
c_0	1.384 ± 0.12	0.716 ± 0.06	0.395 ± 0.04	0.214 ± 0.03	0.077 ± 0.02	0.069 ± 0.02	0.048 ± 0.02	0.047 ± 0.02	0.024 ± 0.02	0.024 ± 0.02
K_{OH}	−0.002 ± 0.5	1.9 ± 6.5	3.56 ± 7.6	−0.0002 ± 0.3	4.38 ± 12	5.3 ± 13	5.0 ± 16	4.52 ± 17	5.5 ± 28	5.0 ± 26
$\Delta G_C/RT$	−1.3 ± 48	−0.03 ± 0.5	−0.008 ± 0.3	−1.6 ± 265	−0.02 ± 0.4	−0.04 ± 0.35	−0.08 ± 0.4	−0.01 ± 0.6	−0.13 ± 0.7	−0.15 ± 0.7
					Eqs. (18) and (19)					
c_0	1.56 ± 0.13	0.86 ± 0.03	0.435 ± 0.07	0.23 ± 0.14	0.081 ± 0.14	0.07 ± 0.1	0.05 ± 0.09	0.051 ± 0.09	0.025 ± 0.06	0.025 ± 0.07
c_1	2.779 ± 0.64	2.8 ± 0.28	3.97 ± 1.4	3.0 ± 4.6	4.67 ± 15	5.3 ± 14	5.1 ± 16	4.5 ± 15	5.3 ± 23	5.2 ± 25
c_2	−0.41 ± 0.03	−0.45 ± 0.01	−0.41 ± 0.04	−0.41 ± 0.19	−0.39 ± 0.39	−0.38 ± 0.3	−0.39 ± 0.36	−0.40 ± 0.39	−0.40 ± 0.49	−0.41 ± 0.55
					Eq. (22)					
c_0	0.208 ± 0.02	0.589 ± 0.04	1.432 ± 0.05	2.164 ± 0.07	3.218 ± 0.05	3.422 ± 0.05	3.716 ± 0.08	3.641 ± 0.08	4.355 ± 0.08	4.395 ± 0.10
c_1	−9.36 ± 0.47	−4.24 ± 0.83	−10.13 ± 1.1	−10.53 ± 1.4	−13.49 ± 0.98	−15.8 ± 1	−14.33 ± 1.5	−12.5 ± 1.6	−13.33 ± 1.7	−13.6 ± 1.9
c_2	−0.303 ± 0.007	−0.22 ± 0.01	−0.299 ± 0.01	−0.327 ± 0.02	−0.349 ± 0.01	−0.385 ± 0.01	−0.36 ± 0.02	−0.329 ± 0.02	−0.342 ± 0.02	−0.356 ± 0.03
c_3	−1.126 ± 0.13	−3.57 ± 0.22	−2.15 ± 0.29	−0.966 ± 0.37	−1.297 ± 0.27	−0.816 ± 0.27	−1.396 ± 0.4	−1.631 ± 0.42	−2.11 ± 0.45	−1.97 ± 0.52
					Eq. (24)					
c_0	−0.725 ± 0.38	−0.098 ± 0.28	0.512 ± 0.38	1.157 ± 0.41	2.142 ± 0.44	2.237 ± 0.48	2.607 ± 0.45	2.628 ± 0.41	3.302 ± 0.43	3.298 ± 0.45
c_1	6.78 ± 9.7	7.64 ± 7.2	5.77 ± 9.6	6.88 ± 10.5	5.11 ± 11.2	4.68 ± 12	4.85 ± 11.6	4.99 ± 10.6	4.88 ± 11	5.34 ± 11.5
c_2	−6.36 ± 1.7	−7.42 ± 1.2	−7.31 ± 1.7	−6.61 ± 1.8	−7.33 ± 1.9	−7.46 ± 2.1	−7.62 ± 2	−7.31 ± 1.8	−8.01 ± 1.9	−8.12 ± 1.98

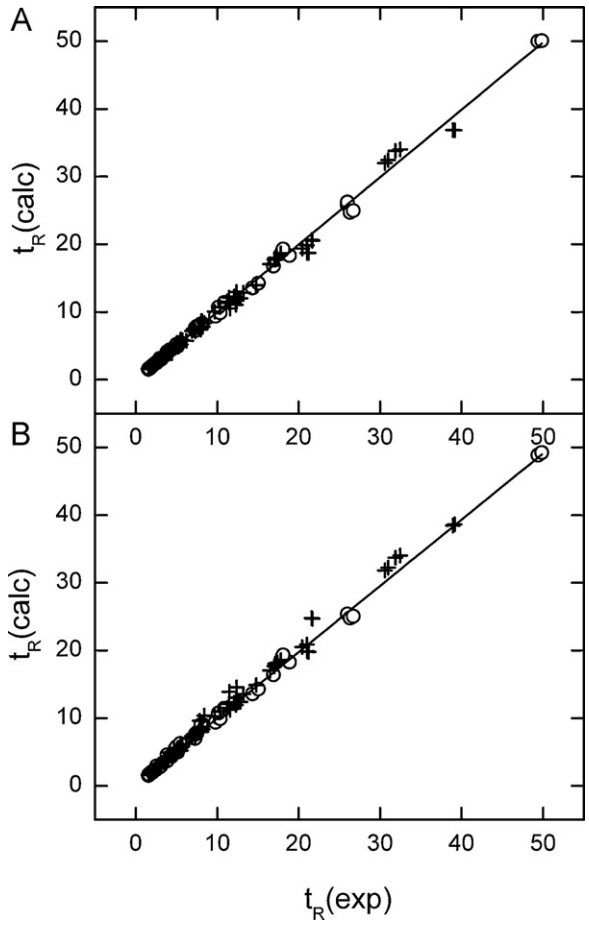

Fig. 1. Calculated from (A) Eqs. (16) and (17) and (B) Eqs. (18) and (19) versus experimental retention times of the amino acids. Circles represent fitting and crosses prediction results.

a maximum at 6.6% and the prediction error increases to 4% with a maximum at 12%. In contrast, Eq. (24) fails completely to fit the data, Eq. (22) gives good fittings but very poor predictions with mean and maximum errors at 11.5% and 43%, respectively, and Eq. (18) exhibits a better performance but the maximum fitting and prediction error is around 20%. The above performance of the proposed models is clearer depicted in Figs. 1 and 2, which show the calculated versus experimental retention times of the amino acids.

It is seen that when retention is governed almost exclusively by dispersion forces, as in the case of alkylbenzenes, all the models developed in the theoretical section can be adopted for solute retention prediction with good results. However, in case of analytes with polar and ionizable groups, like amino acids, that is, when Coulombic forces come into play, only Eq. (16) seems to be the best model.

Note that a minimum of about 5 experiments is needed for a successful prediction of the analytes retention times. However, we should take into consideration that the proposed models are valid in chromatographic systems under variable composition with the meaning that the additive (alkanol) and its concentration can change. That is, we can predict the retention behavior of an analyte in the presence of an additive that was not previously used in the fitting procedure. For example, in the case of alkylbenzenes we can predict the behavior of propanol, pentanol and heptanol, whereas in the case of amino acids we can predict the behavior of propanol, butanol and pentanol.

The objective of this paper is to develop retention models that can effectively predict the retention times of analytes under

Table 7
Absolute mean and maximum percentage error between experimental and calculated retention times.

Fitting equation	Mean	Max	Mean	Max	Mean	Max
	Alkylbenzenes/Nucleosil		Alkylbenzenes/Zorbax SB-C18		Amino acids/Zorbax Eclipse-AAA	
			Fitting error			
(16) and (17)	1.0	2.6	0.6	1.9	2.5	6.6
(18) and (19)	1.0	2.6	0.7	1.9	4.8	18.0
(22)	0.7	1.9	0.3	0.9	0.8	3.7
(24)	1.3	3.5	2.0	5.2	14.4	53.3
			Prediction error			
(16) and (17)	2.8	11.5	2.3	8.4	4.1	10.8
(18) and (19)	2.8	11.1	2.2	8.3	4.7	23.1
(22)	2.1	6.2	1.6	7.6	11.5	43.1
(24)	2.3	9.5	2.6	9.3	7.9	28.2

the effect of aliphatic alcohol additives in reversed-phase liquid chromatography. Such models could be used for prediction and optimization to determine both the best alcohol that should be used as an additive and its concentration. Under isocratic conditions this target is easily attainable by constructing a 2D table in which the resolution R_s is calculated as a function of both n and φ_D, $R_s(n, \varphi_D)$.

As an example of such an application we have applied Eq. (16) to find the optimum conditions for the separation of the mixture of amino acids. From the retention times of Tables 1–3 we observe that the separation of a mixture of the alkylbenzenes we studied presents no difficulties in contrast to the amino acids we used, where there are pairs of analytes that either coelute or elute close to each other. The peak widths, w, necessary for the calculation of the resolution were estimated from the plot of w versus t_R. We found that the peak width of all amino acids in all mobile phases is given by $w = 0.019t_R + 0.05$. Using this expression and t_R values calculated from Eq. (16) we have constructed the 2D table of $R_s(n, \varphi_D)$. Thus for the amino acids an optimum may be when $n = 6$ and $\varphi_D = 0.05$ (last line in Table 3). The resolution values for this optimum are: $R_s(\text{exp}) = 0.9$ and $R_s(\text{calc}) = 0.5$. The chromatogram recorded under these conditions is shown in Fig. 3. For comparison, this figure includes the chromatogram when $n = 4$ and $\varphi_D = 0.05$ ($R_s(\text{exp}) \approx 0$ and $R_s(\text{calc}) = 0.2$). From this figure it is clear that the mixture of 10 amino acids was not able to be separated in 13 min by using BuOH additive in the mobile phase at $\varphi_D = 0.05$, since two pairs of solutes coelute, Met and Trp as well as Leu and Ile. In contrast, when the same content of HexOH instead of BuOH is added in the mobile phase, a perfect resolution of the amino acid mixture is achieved within only 5.5 min. At this point we should stress that the addition of small quantities of aliphatic alcohols does not merely shift the peaks towards smaller retention times but it may change the order of elution. Consequently, a proper manipulation of the mobile phases studied in the present paper may enhance the retention and/or selectivity in RPLC.

To sum up, at least one of the retention models developed in the present study, that of Eq. (16), can be adopted for solute retention prediction with very good results when small quantities of alkanol additives are added in the mobile phase with a high concentration of MeOH as the main organic modifier. The model can also predict the effect of an additive on the retention properties of analytes, even if this additive was not used in experiments for the fitting procedure, provided that it belongs to the same homologous series

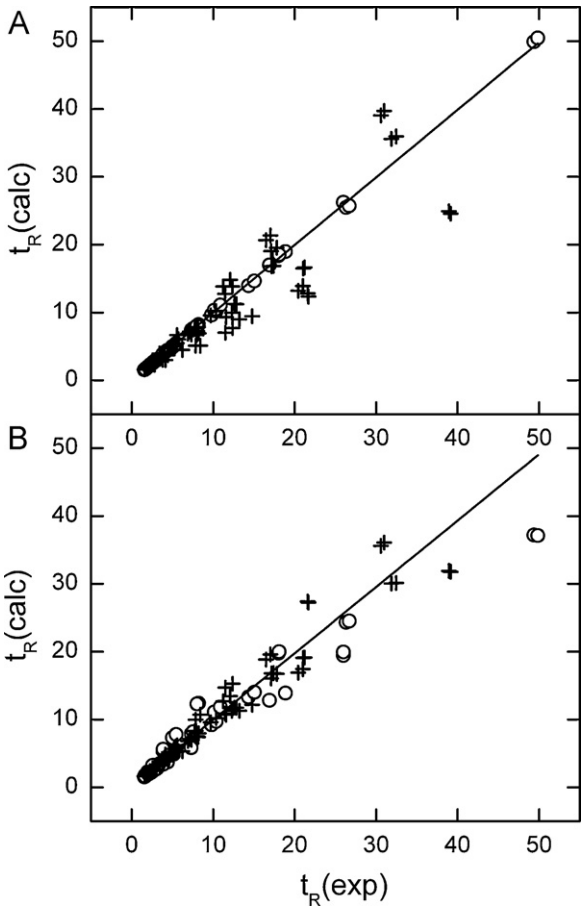

Fig. 2. Calculated from (A) Eq. (22) and (B) Eq. (24) versus experimental retention times of the amino acids. Circles represent fitting and crosses prediction results.

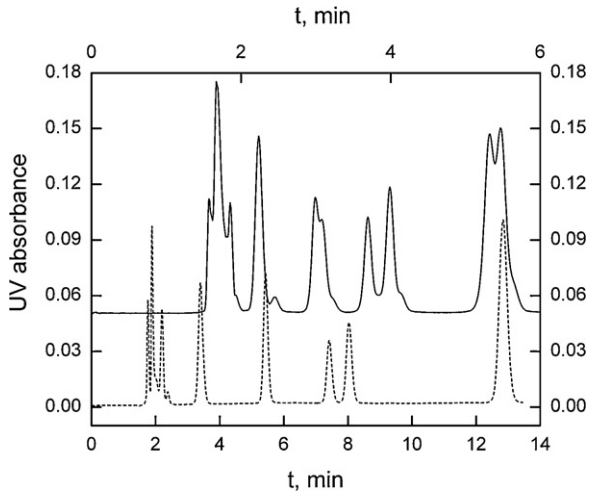

Fig. 3. Chromatograms of a mixture of Arg, Tau, Dopa, Ala, Met, Trp, Phe, Val, Leu and Ile (from left to right) recorded in mobile phases with $\varphi_{\text{MeOH}} = 0.5$ containing BuOH (- - -) or HexOH (—) as an additive at $\varphi_D = 0.05$. Met and Trp as well as Leu and Ile coelute in the chromatogram obtained with BuOH additive in the mobile phase. The bottom x-axis corresponds to the chromatogram recorded in the eluent with BuOH, whereas the top x-axis to that with HexOH.

of alkanols. Finally, the usefulness of such mobile phases in the tuning of RPLC separations was demonstrated.

List of symbols

A	analyte molecule
B	organic modifier molecule
b	slope in Eq. (23)
c_0, c_1, c_2, c_3	coefficients of $\ln k$ expressions
D	additive molecule
K	equilibrium constant of Eq. (3)
k	retention factor of the sample solute
m	as superscript it denotes the mobile phase
N	number of data
n	number of carbon atoms of an alcohol
P	= AD, an associate
R_s	resolution
S	the mixture of constant composition of water and organic modifier
s	as superscript it denotes the stationary phase
t_R	elution time of a solute
t_0	column hold-up time
x_1, x_2	factors, separation variables
W	water molecule
w	weighting factor

Greek letters

β	$= \beta_D = K$. It is expressed by means of Eq. (19)
β_A, β_D	equilibrium constants of the Langmuir isotherms, Eqs. (10) and (11)
ΔG	free energy due to A–D interactions
ΔG_{OH}	free energy due to the interaction of A with the hydroxyl group of an alkanol
ΔG_C	free energy due interactions of A with each of the carbon groups of an alkanol
ΔG_{ads}	free energy of adsorption of an additive alcohol
$\Delta G_{a,OH}$	free energy of adsorption due to interactions of the hydroxyl group of an alcohol with the adsorbing surface
$\Delta G_{a,C}$	free energy of adsorption due to interactions of each of the carbon groups of an alcohol with the adsorbing surface
Φ	phase ratio
φ	volume fraction
φ_A^s	volume fraction of A in the stationary phase
φ_A^m	volume fraction of A in mobile phase
$\varphi_D^m = \varphi_D$	volume fraction of D in the mobile phase
φ_P^m	volume fraction of associate P (=AD) in the mobile phase

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