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Retention model for singly and doubly charged analytes in ion-interaction chromatography

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Abstract

A theoretical model for −1 and −2 charged analytes in ion-interaction chromatography was studied and a general equation was developed for describing the simultaneous effect on the concentrations of the ion-interaction reagent, organic modifier and counter ion on k'. The modelling concerns the retention process as a result of several adsorption and ion-exchange equilibria, considering the adsorption of neutral and charged ion pairs on the stationary phase. The retention model was applied to a large number of experimental chromatographic data obtained in the separation of metal complexes on a silica C_8 column. The experimental design was planned in order to describe the chromatographic behaviour in a multi-dimensional space (k' versus tetrabutylammonium, nitrate and methanol concentrations). The adsorption and ion-exchange constants were calculated by an iterative method of non-linear regression. The average error between the calculated and experimental k' values (<13%) is close to the experimental error (about 5%), supporting the applicability of the equation developed.

Keywords: Retention models; Thermodynamic parameters; Metal complexes; Acid Alizarin Violet N; Cobalt complexes; Copper complexes

1. Introduction

The theories which are able to describe quantitatively analyte retention behaviour in ion-interaction chromatography include thermodynamic [1–10] and stoichiometric approaches [11–22]. As demonstrated in the last 20 years, both methods are useful for obtaining numerical equations in order to clarify the mechanisms involved and for their modelling. In fact, these two methods do not have fundamental differences, because stoichiometric models, based on adsorption and ion-exchange equilibria, utilize the equilibrium constants which represent and include thermodynamic parameters, e.g., the free energy of adsorption of the molecule on the stationary phase and the electrostatic potential of the surface.

Thermodynamic methods, including electrostatic theories [2,4,10], have generally been used

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for retention modelling as a function of single parameters, generally the concentration of the ion-interaction reagent, but also salt concentration [8] and percentage of organic modifier [23]. Few studies to combine the simultaneous effect of ion-interaction reagent concentration with percentage of organic modifier have been reported [3,24].

Since many predictions of electrostatic theories are not fully in agreement with the experimental data, they have been modified with modern theories of surfactants based on a multi-site occupancy model [9]. On the other hand, the stoichiometric models have been used and verified by different approaches; some workers have utilized their models to describe the experimental results mainly in a qualitative way [12,21,22]. In most works, the validity of the models proposed is based on regressions which consider one variable at a time, e.g., organic modifier [19], ion interaction reagent [13–19] or salt [11].

Several workers have considered the separation of different charged analytes in ion-interaction chromatography [25–31]. Attention has mainly been focused on the behaviour of sulphonates with eluents containing octylamine [25] or, more generally, alkylammonium as an ion-interaction reagent. The separation of charged metal complexes has also been studied [30,31]. In no case has the chromatographic behaviour of different charged analytes been described in order to predict retention changes of a charged solute when varying simultaneously all the principal experimental parameters (e.g., concentration of ion-interaction reagent, percentage of organic modifier and concentration of salt).

In this work, a theoretical model for -1 and -2 charged analytes (Cu^{2+} and Co^{2+} complexed with Acid Alizarin Violet N) was studied and a general equation was developed, which describes the simultaneous effect of the concentrations of ion-interaction reagent, organic modifier and salt on k'. A multi-variable regression analysis allows the calculation of the constants of the equation and, therefore, the use of the model as a tool for predicting retention behaviour over a wide concentration range of the eluent components considered.

2. Experimental

2.1. Apparatus

The chromatographic system used was a Varian (Walnut Creek, CA, USA) LC 9010 liquid chromatograph equipped with a 100-μl sample loop, a Kontron (Milan, Italy) UV–Vis spectrophotometric detector and a Axxiom Chromatography (Calabasas, CA, USA) Model 727 data system. The separation column was LiChrosorb RP-8 (10 μm) (250 × 4 mm I.D.), coupled with a LiChroCART 100 RP-8 (5 μm) guard column (4 × 4 mm I.D.), both obtained from Merck (Darmstadt, Germany). An Orion (Cambridge, MA, USA) digital pH meter was used for pH measurements.

2.2. Reagents

4-Hydroxy-3-(2-hydroxynaphthylazo)benzenesulphonic acid (Acid Alizarin Violet N) was obtained from Aldrich (Milwaukee, WI, USA) and tetrabutylammonium hydroxide (TBA) from Fluka (Buchs, Switzerland). Acetic acid, sodium hydroxide, sodium nitrate, methanol and standard metal solutions (Cu^{2+} and Co^{2+} at 1000 mg/l) were Merck analytical-reagent grade products.

2.3. Procedures

Columns and tubings were cleaned daily with methanol–water (80:20, v/v) solution for 30 min and with pure methanol for 10 min at a flow-rate of 1.0 ml/min. Eluents were prepared daily with high-purity water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) and contained tetrabutylammonium hydroxide, sodium nitrate, acetate buffer (40 mM), methanol as required (see below) and 1.6 μM Acid Alizarin Violet N, in order to prevent dissociation of chelates. The aqueous pH was adjusted to 5.5 by adding NaOH. Eluents were filtered through a 0.45-μm membrane filter (Millipore HAWP 04700) and degassed under vacuum before use.

Columns were then conditioned with the mobile phase solution. Standard metal solutions were obtained by diluting suitable volumes of the

metal stock solutions with 1.0 m*M* Acid Alizarin Violet N. The pH was adjusted in order to match that of the eluent. The eluent flow-rate was 1.0 ml/min and the wavelength for detection was chosen as 270 nm owing to the absorption behaviour of the chelates and ligand. The column dead volume, determined from the unretained peak of water, was 2.8 ml for the chromatographic conditions chosen.

2.4. Multi-variable regression

Calculations and graphic elaborations were performed using a 486 SX IBM-compatible PC with the software Sigma-Plot for Windows (Jandell Scientific). The multi-variable non-linear regression analysis is based on the Marquandt–Levenberg algorithm and allows the determination of the equation parameters by iterative calculations.

3. Theoretical

The equilibria involved in the retention mechanism were considered for species obtained by interaction of Q^+ with X^{2-}, where Q^+ is the ion-interaction reagent and X^{2-} is a doubly negatively charged analyte. The retention model was derived taking into account simultaneous effects of the ion-interaction reagent, organic modifier and counter ion (C^-) present in the mobile phase.

As shown in Fig. 1, the retention mechanism

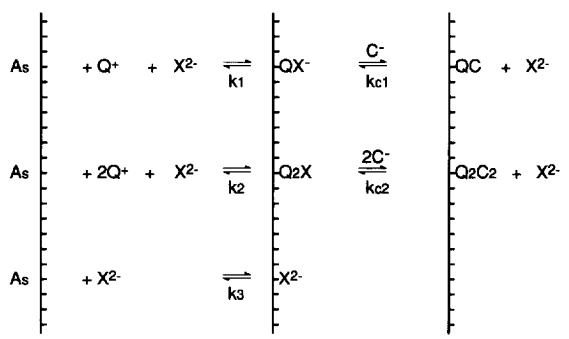

Fig. 1. Equilibria in the column. Q^+ = ion-interaction reagent; X^{2-} = lipophilic analyte ion; C^- = counter ion; A_s = available sites on stationary phase.

can be considered to be the result of the contributions of two main processes: adsorption of pair of ions, not necessarily ion pairs, on the stationary phase, and ion exchange between the anion of the ion pairs (X^{2-}) and other counter ions (C^-) of the mobile phase.

3.1. Adsorption equilibria

Ion-pair reagent Q^+, added to the mobile phase, is adsorbed, as ion pairs with the analyte, on stationary phase. The stoichiometric approach makes it necessary to consider every equilibrium that the analyte can undergo in its retention process in the chromatographic column. In fact, the adsorption of ion pairs can pass through the formation of charged and neutral ion pairs (QX^- and Q_2X, respectively). The resulting equilibria and their constants are

$$A_s + [Q^+] + [X^{2-}] \overset{K_1}{\rightleftharpoons} (QX^-)$$

$$K_1 = \frac{(QX^-)}{A_s[Q^+][X^{2-}]} \quad (1)$$

$$A_s + 2[Q^+] + [X^{2-}] \overset{K_2}{\rightleftharpoons} (Q_2X)$$

$$K_2 = \frac{(Q_2X)}{A_s[Q^+]^2[X^{2-}]} \quad (2)$$

where A_s is the number of free adsorption sites on the lipophilic stationary phase, according to Xianren and Baeyens [22], and the round and the square brackets refer to the stationary and mobile phases, respectively.

Among the interactions between the different existing species and the stationary phase, the partition process of the analyte between the mobile and stationary phases in the absence of an ion-pair reagent (reversed-phase retention mechanism) cannot be excluded. The corresponding equilibrium (and its constant) is

$$A_s + [X^{2-}] \overset{K_3}{\rightleftharpoons} (X^{2-}) \quad K_3 = \frac{(X^{2-})}{A_s[X^{2-}]} \quad (3)$$

Such an equilibrium can be relevant when part of the molecular structure of the analyte is highly lipophilic.

3.2. Ion-exchange equilibria

Every adsorbed ion pair can exchange its anion with the others present in the mobile phase. For a general ion C^-, the equilibria involved and their constants are

$$(QX^-) + [C^-] \overset{K_{c1}}{\rightleftharpoons} (QC) + [X^{2-}]$$

$$K_{c1} = \frac{(QC)[X^{2-}]}{(QX^-)[C^-]} \tag{4}$$

$$(Q_2X) + 2[C^-] \overset{K_{c2}}{\rightleftharpoons} (Q_2C_2) + [X^{2-}]$$

$$K_{c2} = \frac{(Q_2C_2)[X^{2-}]}{(Q_2X)[C^-]^2} \tag{5}$$

Introducing the adsorption capacity of the column k_0, defined as the amount of adsorbed species and free sites still available [22], Eq. 6 can be derived:

$$k_0 = A_s + (Q_2X) + (QX^-) + (X^{2-}) + (QC) + (Q_2C_2) \tag{6}$$

Obtaining A_s from the adsorption equilibrium 2:

$$A_s = \frac{1}{K_2[Q^+]^2}\frac{(Q_2X)}{[X^{2-}]}$$

(QX^-) from the adsorption equilibrium 1:

$$(QX^-) = A_sK_1[Q^+][X^{2-}] = \frac{K_1}{K_2[Q^+]}(Q_2X)$$

(X^{2-}) from the partition equilibrium 3:

$$(X^{2-}) = A_sK_3[X^{2-}] = \frac{K_3}{K_2[Q^+]^2}(Q_2X)$$

(QC) from the ion-exchange equilibrium 4:

$$(QC) = \frac{K_{c1}(QX^-)[C^-]}{[X^{2-}]} = K_{c1}\cdot\frac{K_1}{K_2}\frac{[C^-]}{[Q^+]}\frac{(Q_2X)}{[X^{2-}]}$$

and (Q_2C_2) from the ion-exchange equilibrium 5:

$$(Q_2C_2) = K_{c2}[C^-]^2\frac{(Q_2X)}{[X^{2-}]}$$

it is possible to rewrite Eq. 6 as

$$k_0 = \frac{(Q_2X)}{[X^{2-}]}\left\{\frac{1}{K_2[Q^+]^2} + [X^{2-}] + \frac{K_1}{K_2}\frac{[X^{2-}]}{[Q^+]} + \frac{K_3}{K_2}\frac{[X^{2-}]}{[Q^+]^2} + \frac{K_{c1}K_1}{K_2}\frac{[C^-]}{[Q^+]} + K_{c2}[C^-]^2\right\}$$

from which is easily obtained

$$\frac{(Q_2X)}{[X^{2-}]} = \frac{k_0K_2[Q^+]^2}{\{1 + K_2[Q^+]^2[X^{2-}] + K_1[Q^+][X^{2-}] + K_3[X^{2-}] + K_{c1}K_1[Q^+][C^-] + K_2K_{c2}[Q^+]^2[C^-]^2\}} \tag{7}$$

Eq. 8 is the definition of the capacity factor k' for the solute ion X^{2-}:

$$k' = \phi K_d = \phi\cdot\frac{(Q_2X) + (QX^-) + (X^{2-})}{[X^{2-}]} \tag{8}$$

where ϕ = phase ratio and K_d = distribution coefficient.

Substituting (Q_2X), (QX^-) and (X^{2-}), obtained previously, in Eq. 8, k' can be written as

$$k' = \phi\left\{\frac{(Q_2X)}{[X^{2-}]} + \frac{(QX^-)}{[X^{2-}]} + \frac{(X^{2-})}{[X^{2-}]}\right\} = \phi\frac{(Q_2X)}{[X^{2-}]}\left\{\frac{K_2[Q^+]^2 + K_1[Q^+] + K_3}{K_2[Q^+]^2}\right\} \tag{9}$$

Introducing the ratio $(Q_2X)/[X^{2-}]$, as expressed by Eq. 7, in Eq. 9, the following equation is obtained:

$$k' = \phi k_0\cdot\frac{K_2[Q^+]^2 + K_1[Q^+] + K_3}{\{1 + K_2[Q^+]^2[X^{2-}] + K_1[Q^+][X^{2-}] + K_3[X^{2-}] + K_1K_{c1}[Q^+][C^-] + K_2K_{c2}[Q^+]^2[C^-]^2\}} \tag{10}$$

Eq. 8 (from which Eq. 10 is derived) is equiva-

lent to the expression of k' introduced by the electrostatic theory:

$$k' = \phi \exp\left(\frac{-\Delta G_B^\circ - z_B F \Delta\Psi_0}{RT}\right)$$

According to this theory, the equilibrium distribution of an analyte ion, B, results primarily determined by a chemical energy term, $-\Delta G_B^\circ$, and by an electrostatic energy term, $-z_B F \Delta\Psi_0$ [2,4,8]. $\Delta\Psi_0$ is dependent on the concentration of the ion-pair reagent and the dielectric constant and the ionic strength of the mobile phase. In our approach, such contributions are expressed by the second and the third terms in Eq. 10 for a given solvent.

The contribution of the organic modifier in the expression of k' for ion-interaction chromatography has also been considered.

The effect of the organic modifier on retention times has been studied by several workers [3,23,24,32,33]. A combination of a thermodynamic method with an empirical relationship [23] was used to derive the following expression, based on the electrostatic model [24]:

$$k' = ab\mathrm{e}^{c\varphi} \tag{11}$$

where a is a function of the mobile phase composition, b and c ($c < 0$) are constant for a given ion-pair reagent–organic modifier combination and for each solute, and φ is the concentration of the organic modifier. In our study, the term that accounts for effects of mobile phase compositions (ion-pair reagent and counter ion concentrations) is expressed by the third term in Eq. 10.

Introducing this term in Eq. 11, the following relationship is finally obtained:

$$k'_{X^{2-}} = be^{c\varphi} \cdot \frac{K_2[Q^+]^2 + K_1[Q^+] + K_3}{\{1 + K_2[Q^+]^2[X^{2-}] + K_1[Q^+][X^{2-}] + K_3[X^{2-}] + K_1K_{c1}[Q^+][C^-] + K_2K_{c2}[Q^+]^2[C^-]^2\}} \tag{12}$$

The constant $k_0\phi$ has been included in the constant b. Eq. 12 is the expression obtained for the retention behaviour of doubly charged analytes in ion-interaction chromatography in the presence of an ion-interaction reagent and counter ion in an organic–water mixture.

The retention behaviour for a singly charged analyte is also described by Eq. 12 (K_2 and K_{c2} = zero), which becomes

$$k'_{X^-} = be^{c\phi} \cdot \frac{K_1[Q^+] + K_3}{1 + K_1[Q^+][X^-] + K_3[X^-] + K_1K_{c1}[Q^+][C^-]} \tag{13}$$

4. Results and discussion

The retention model derived was tested for complexes of metal ions (Cu^{2+} and Co^{2+}) with Acid Alizarin Violet N, a monosulphonated azo dye. According to our previous studies [31,34], the Cu–ligand complex has a single negative charge (metal to ligand ratio 1:1), whereas the Co–ligand complex has a double negative charge (metal to ligand ratio 1:2).

The experimental design was planned in order to describe the chromatographic behaviour in a multi-dimensional space: k' versus concentration of tetrabutylammonium (as ion-interaction reagent), nitrate (as counter ion) and methanol (as organic modifier). The concentrations of NO_3^-, TBA and CH_3OH in the eluent were varied over a relatively wide range, obtaining 76 and 73 retention data for Cu and Co complexes, respectively.

Eq. 12 was tested by multi-variable non-linear regression. The analyte concentrations used in the calculations were $7.86 \cdot 10^{-5}$ M for the Cu complex and $8.48 \cdot 10^{-5}$ M for the Co complex, which represent the actual amounts injected into the separation column during the experimental work. Since, for small amounts of sample injected, the capacity factor does not change appreciably when the sample size is varied [35], in the present work the effect of this parameter was not studied.

On the basis of Eq. 12, from the experimental

Table 1
Metal-specific constants (K_1, K_2, K_3, K_{c1}, K_{c2}, b, c) for Co–ligand and Cu–ligand complexes

Constant	Co–ligand complex (X^{2-})[a]	Cu–ligand complex (X^-)[b]	Cu–ligand complex (X^-)[a]
K_1	$9.87 \cdot 10^4$	$6.77 \cdot 10^3$	$6.77 \cdot 10^3$
K_2	$1.34 \cdot 10^7$	–	$8.38 \cdot 10^{-4}$
K_3	28.0	24.6	24.6
K_{c1}	$1.75 \cdot 10^{-2}$	$3.90 \cdot 10^{-2}$	$3.90 \cdot 10^{-2}$
K_{c2}	$9.83 \cdot 10^{-12}$	–	$3.90 \cdot 10^{-2}$
b	$3.38 \cdot 10^4$	73.6	72.4
c	−27.5	−15.3	−15.3

[a] Values obtained by application of Eq. 12.
[b] Values obtained by application of Eq. 13.

data, the adsorption constants K_1, K_2 and K_3, the ion-exchange constants K_{c1} and K_{c2} and the other parameters b and c were determined, for each analyte, by iterative calculations. Table 1 shows the results of this process for the metal complexes studied. For the Cu complex, both Eqs 12 and 13 were tested. The similar values of K_1, K_3, K_{c1}, b and c (see Table 1) and the similar predicted k' obtained from the application of both kinds of equations show that the weight of the terms including K_2 and K_{c2} is negligible when Eq. 12 is applied, which means that Eq. 12 reduces to Eq. 13. Therefore, the retention behaviour of the Cu complex can be well described either by Eq. 12 (and its constants) or by Eq. 13.

Among the adsorption constants of the Co complex, K_1 is lower than K_2 by about two orders of magnitude, in agreement with the greater contribution of the uncharged ion pair in the retention mechanism. The constant that represents the adsorption of the analyte, K_3, has a low value compared with K_1 and K_2, showing that the interaction of the analyte with the ion-interaction reagent is dominant. Anyway, it should be pointed out that equilibrium 3 allows even the chromatographic data obtained at [TBA] = 0 to be included in the iterative calculation. The difference between the ion-exchange constants, instead, suggests that the charged ion pair QX^- provides a significant contribution to the counter ion effect.

For the Cu complex, the adsorption constant K_1 is smaller than that for the Co complex; this supports the greater affinity for the stationary phase of Co species (owing to its pronounced lipophilicity) than Cu. As previously observed for Co, the retention of the analyte is not predominantly due to the adsorption equilibrium 3. The values of K_{c1} for Cu and Co species are of the same order of magnitude, suggesting the same tendency of the ion pairs to exchange the analyte with the nitrate counter ion.

Concerning the values of b and c, it should be noted that they are characteristic of the solute considered. The parameter c is negative, which means that the capacity factor of a solute decreases with increasing organic modifier concentration; a larger absolute value of c means that there is a stronger effect of the percentage of the organic modifier on the retention of the solute (the retention behaviour of the Co complex is influenced more than that of the Cu complex by an increase in methanol concentration).

Using the values of the constants obtained, predictions can be made for the capacity factors of each analyte ion at different eluent compositions. Table 2 shows the individual deviations between the measured (k'_{meas}) and predicted (calculated, k'_{calc}) capacity factors for the Co and Cu complexes. The errors are given as percentage values of the relative differences, according to the expression

$$\text{error}\,(\%) = \frac{|k'_{meas} - k'_{calc}|}{k'_{meas}} \cdot 100$$

Table 2
Comparison of observed and predicted capacity factors for Cu and Co complexes with Acid Alizarin Violet N (AVN)

Eluent			Cu–AVN			Co–AVN		
Nitrate (*M*)	Methanol (%)	TBA (*M*)	k'_{meas}	k'_{calc}	Error (%)	k'_{meas}	k'_{calc}	Error (%)
0.0000	58.0	0.0120	1.03	1.07	4.1	7.86	9.85	25.4
0.0000	60.0	0.0120	0.87	0.79	9.2	6.08	5.68	6.5
0.0120	45.2	0.0120	7.59	7.31	3.7	–	–	–
0.0120	48.0	0.0120	4.91	4.76	3.0	–	–	–
0.0120	50.0	0.0120	2.90	3.51	21.0	–	–	–
0.0120	54.8	0.0120	1.70	1.68	0.9	26.10	19.86	23.9
0.0120	58.0	0.0120	1.03	1.03	0.3	7.45	8.23	10.5
0.0120	60.8	0.0120	0.80	0.67	15.8	4.75	3.81	19.8
0.0220	55.0	0.0120	1.55	1.59	2.3	16.32	16.53	1.3
0.0233	57.0	0.0120	1.27	1.16	8.4	10.67	9.39	12.0
0.0238	58.0	0.0120	0.92	1.00	8.4	5.79	7.09	22.4
0.0250	60.0	0.0120	0.74	0.73	1.1	4.50	4.03	10.4
0.0476	58.0	0.0120	0.90	0.93	3.6	5.18	5.54	6.9
0.0500	60.0	0.0120	0.73	0.68	6.5	3.31	3.12	5.6
0.0510	57.0	0.0120	1.05	1.08	2.5	6.63	7.07	6.6
0.0722	55.0	0.0120	1.25	1.38	10.6	9.70	10.30	6.2
0.1000	55.0	0.0120	1.21	1.29	6.6	8.90	8.53	4.2
0.1000	60.0	0.0120	0.69	0.60	12.9	2.56	2.15	15.9
0.1010	58.0	0.0120	0.77	0.81	5.7	3.32	3.71	11.8
0.1020	57.0	0.0120	0.91	0.95	3.9	4.89	4.86	0.7
0.1490	57.0	0.0120	0.86	0.85	1.1	3.91	3.77	3.6
0.1500	55.0	0.0120	1.08	1.15	6.7	6.50	6.51	0.1
0.1500	60.0	0.0120	0.65	0.54	17.4	2.20	1.64	25.3
0.1778	55.0	0.0120	0.99	1.09	9.9	5.23	5.75	9.9
0.2000	60.0	0.0120	0.63	0.48	23.0	1.50	1.33	11.4
0.2024	58.0	0.0120	0.71	0.66	7.7	2.38	2.28	4.1
0.2500	60.0	0.0120	0.65	0.44	32.0	1.57	1.12	29.0
0.0120	60.0	0.0000	0.26	0.18	29.0	0.12	0.06	46.8
0.0120	60.0	0.0030	0.54	0.33	38.2	1.36	0.93	32.0
0.0120	60.0	0.0060	0.66	0.48	27.4	1.95	2.07	6.3
0.0120	60.0	0.0090	0.79	0.62	21.3	3.48	3.38	2.9
0.0120	60.0	0.0120	0.83	0.76	8.3	4.36	4.75	8.9
0.0120	60.0	0.0150	1.06	0.90	15.3	6.28	6.11	2.6
0.0120	60.0	0.0180	1.06	1.03	2.7	7.29	7.43	1.9
0.0120	60.0	0.0210	1.19	1.16	2.3	9.31	8.67	6.8
0.0120	60.0	0.0240	1.18	1.29	9.4	10.43	9.83	5.7
0.0250	60.0	0.0160	0.88	0.90	1.8	5.32	5.45	2.4
0.0250	60.0	0.0220	1.09	1.13	3.4	7.79	7.40	5.0
0.0500	60.0	0.0160	0.77	0.82	6.3	3.96	4.11	3.7
0.0500	60.0	0.0220	0.90	1.00	11.2	4.97	5.47	10.0
0.1000	60.0	0.0160	0.73	0.70	4.4	3.03	2.75	9.2
0.1000	60.0	0.0220	0.84	0.82	2.5	4.28	3.59	16.1
0.1500	60.0	0.0160	0.68	0.61	10.6	2.27	2.07	8.8
0.1500	60.0	0.0220	0.70	0.69	1.1	2.50	2.67	7.0
0.2000	60.0	0.0160	0.65	0.54	17.1	2.02	1.66	17.9
0.2000	60.0	0.0220	0.71	0.60	15.5	2.70	2.13	21.1
0.0120	58.0	0.0029	0.66	0.45	32.4	2.51	1.20	52.1
0.0120	58.0	0.0063	0.83	0.67	19.3	3.94	3.81	3.3

Table 2 (*continued*)

Eluent			Cu–AVN			Co–AVN		
Nitrate (*M*)	Methanol (%)	TBA (*M*)	k'_{meas}	k'_{calc}	Error (%)	k'_{meas}	k'_{calc}	Error (%)
0.0120	58.0	0.0091	0.91	0.85	6.3	5.74	5.97	4.0
0.0120	58.0	0.0150	1.25	1.22	2.5	10.37	10.60	2.2
0.0120	58.0	0.0183	1.32	1.42	7.4	11.89	13.11	10.2
0.0120	58.0	0.0210	1.53	1.58	3.1	16.35	15.04	8.0
0.0120	62.0	0.0090	0.58	0.46	21.1	1.95	1.95	0.0
0.0120	62.0	0.0057	0.48	0.34	28.7	1.33	1.12	15.5
0.0120	62.0	0.0183	0.84	0.77	8.4	4.66	4.36	6.4
0.0120	62.0	0.0208	0.92	0.85	7.6	5.69	4.96	12.9
0.0120	62.0	0.0152	0.69	0.67	3.4	3.30	3.57	8.1
0.0120	55.0	0.0032	0.89	0.74	17.2	4.68	3.94	15.9
0.0120	55.0	0.0059	0.99	1.02	2.6	5.64	7.99	41.7
0.0120	55.0	0.0090	1.40	1.33	4.7	13.63	13.38	1.9
0.0120	55.0	0.0150	1.71	1.93	12.7	20.23	24.20	19.6
0.0420	56.0	0.0040	0.74	0.68	7.7	2.75	3.26	18.6
0.0420	61.0	0.0040	0.42	0.32	24.2	0.84	0.82	1.9
0.0420	56.0	0.0200	1.53	1.80	17.5	16.11	16.48	2.3
0.2080	56.0	0.0200	1.03	1.05	2.0	5.50	5.75	4.6
0.2080	61.0	0.0200	0.66	0.49	25.9	1.95	1.45	25.5
0.2080	56.0	0.0040	0.68	0.59	13.9	1.70	1.76	3.4
0.2080	61.0	0.0040	0.37	0.27	26.3	0.81	0.44	45.2
0.0420	61.0	0.0200	0.81	0.84	3.3	4.10	4.16	1.5
0.0120	45.2	0.0016	2.25	2.54	12.9	28.67	28.11	2.0
0.0120	48.0	0.0016	1.68	1.66	1.5	12.25	13.01	6.2
0.0120	50.0	0.0016	1.22	1.22	0.1	6.46	7.50	16.1
0.0120	54.8	0.0016	0.71	0.59	17.5	2.14	2.00	6.4
0.0120	58.0	0.0016	0.52	0.36	31.0	1.39	0.83	40.3
0.0120	60.8	0.0016	0.39	0.23	40.0	0.65	0.38	40.9
0.0120	64.8	0.0016	0.24	0.13	47.1	0.22	0.13	41.9
					Mean 11.9			Mean 13.0

The mean of the errors is given at the bottom of Table 2. It can be noted that the predicted k' values are in accordance with those obtained experimentally as the mean of the errors in both cases does not exceed 13%. This value represents a good result compared with the experimental error of about 5%. The greatest errors for both analytes are generally obtained at low tetrabutylammonium or high nitrate concentrations. This phenomenon is probably the consequence of ignored effects such as the competitive effect of Na^+ (increasing with increasing salt concentration) in the interaction between the analyte and the TBA ion-interaction reagent. As the presence of Na^+ was not considered in the derivation of the retention model, complete agreement between the experimental and calculated k' values could not be observed for eluents containing high concentrations of $NaNO_3$ and small amounts of TBA. However, the model gives good predictions of capacity factors over a relatively wide range of concentrations of the variables studied, covering the usual working conditions.

Figs. 2 and 3 show the retention surfaces (meshes) for the metal complexes studied in this work, calculated according to Eq. 12. The experimental chromatographic results are also plotted (closed circles).

Fig. 2a shows the variation of k' for the Co–

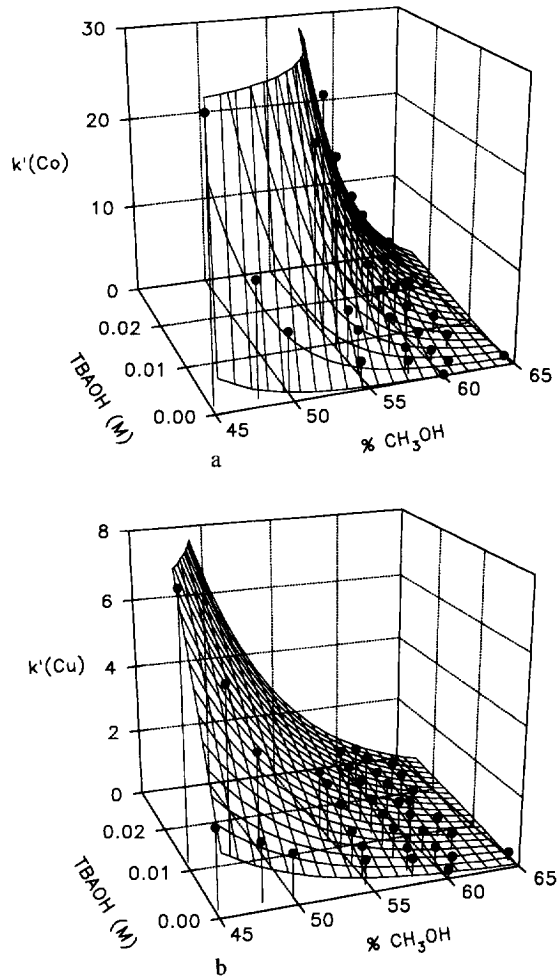

Fig. 2. Calculated retention surface and experimental data for (a) Co–Acid Alizarin Violet N and (b) Cu–Acid Alizarin Violet N complexes. Mobile phase composition: 40 m*M* CH_3COOH–12 m*M* $NaNO_3$–1.6 μM Acid Alizarin Violet N–NaOH up to pH 5.5, with methanol and TBAOH as shown. Detection wavelength 270 nm; flow-rate, 1.0 ml/min; sample volume, 100 μl.

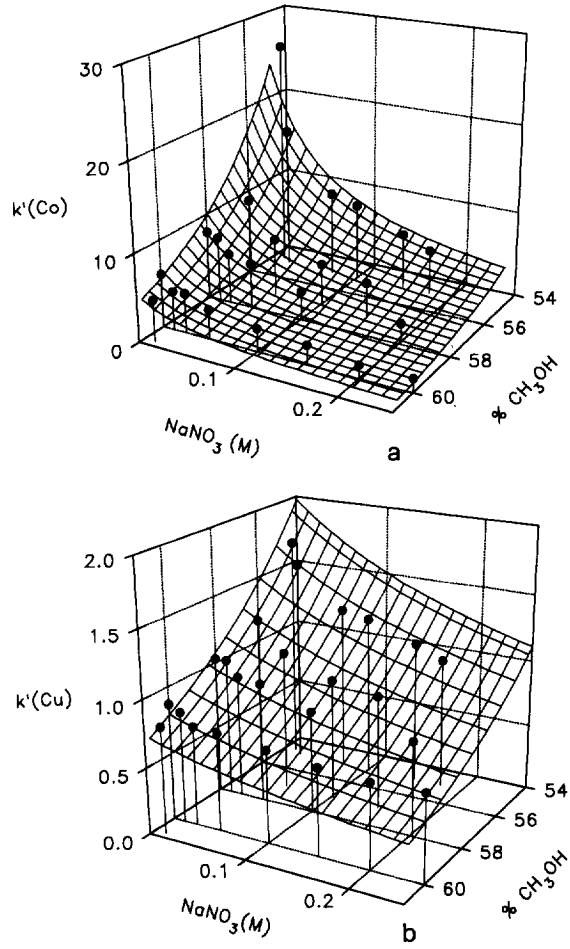

Fig. 3. Calculated retention surface and experimental data for (a) Co–Acid Alizarin Violet N and (b) Cu–Acid Alizarin Violet N complexes. Mobile phase composition: 12 m*M* TBAOH–40 m*M* CH_3COOH–1.6 μM Acid Alizarin Violet N–NaOH up to pH 5.5, with methanol and $NaNO_3$ as shown. Other conditions as in Fig. 2.

ligand complex with different methanol and TBA concentrations at a constant concentration of nitrate ion (0.012 *M*). As predicted by the *k'* expression, higher concentrations of methanol enhance the eluent strength, leading to lower capacity factors. A similar dependence is illustrated in Fig. 2b for the Cu complex.

Fig. 3a (Co) and b (Cu) show the application of the retention model at a constant concentration of TBA in mobile phase (0.012 *M*). The retention of the complexes is well represented by the proposed equation. An increase in $NaNO_3$ concentration results in lower *k'* values, as predicted by Eq. 12.

A comparison was also made between the chromatographic behaviours of Cu–ligand and Co–ligand complexes. The retention surfaces of both metal ions obtained at a constant percentage of methanol (60%) are shown in Fig. 4. It should be noted that for the same mobile phase composition, the capacity factors of the Cu–lig-

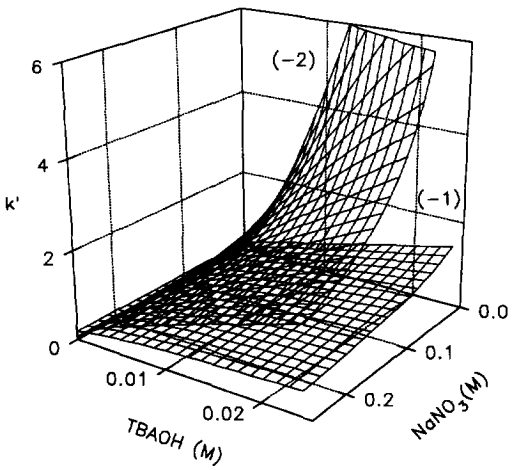

Fig. 4. Comparison of retention of Cu–ligand and Co–ligand complexes vs. TBAOH and $NaNO_3$ concentrations. Mobile phase composition: 60% CH_3OH–40 m*M* CH_3COOH–1.6 μM Acid Alizarin Violet N–NaOH up to pH 5.5, with $NaNO_3$ and TBAOH as shown. Other conditions as in Figure 2.

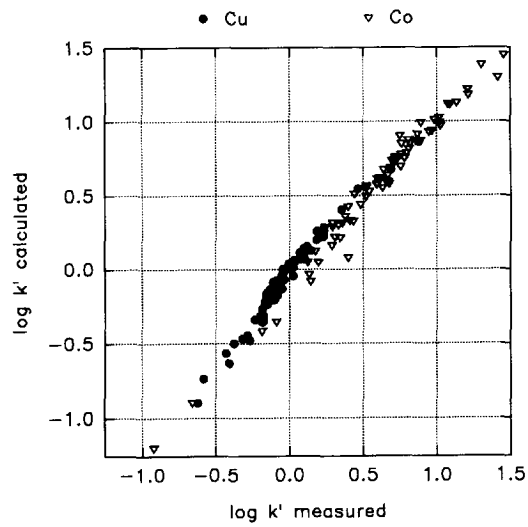

Fig. 5. Relationship between measured and calculated capacity factors for the metal ions studied (149 data pairs plotted).

and complex are lower than those for the Co–ligand complex.

5. Conclusions

This study allowed the development of a theoretical retention model for data prediction in ion-interaction chromatography that accounts for the effects of ion-pair reagent, counter ion and organic modifier concentrations on chromatographic behaviour.

The reliability and applicability of the proposed model for both the prediction and interpretation of retention behaviour of singly and doubly charged solute ions are also supported by the good agreement between the measured and calculated capacity factors for the metal ions studied (Fig. 5). The slope of the log k'_{calc} vs. log k'_{meas} plot is 1.078. The correlation coefficient calculated for the 149 data pairs is 0.990.

From the overall discussion, it can be concluded that, although not all physico-chemical aspects are included, our model represents a useful approach for a theoretical description of the mechanism of ion-interaction chromatography.

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