Preliminary tests to select operating conditions for the accurate determination of stability constants by cation-exchange chromatography: the $Cd^{2+}-Cl^-$ and $Cd^{2+}-NO_3^-$ systems

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Abstract

Ion chromatography (IC) has been demonstrated to be a powerful tool for equilibrium constant determination, related to various cation–ligand systems. Nevertheless, no systematic research has been carried out to develop preliminary checks in order to verify whether the variation in ligand concentration in the eluent at constant ionic strength affects the exchange mechanism for the system of interest in the selected chromatographic column. In this paper, tests are proposed which allow one to determine beforehand the experimental conditions to be used in cation-exchange chromatography, whereby parallel mechanisms of elution (mainly in the reversed-phase mode) are avoided. In this way IC becomes an independent rather than an auxiliary means to obtain accurate β_i values. Cd(II)–Cl$^-$ and Cd(II)–NO$_3$ systems were considered and are discussed.

1. Introduction

Haddad and Foley [1] described a chromatographic retention model for metal ions that simultaneously participate in heterogeneous cation exchange and in homogeneous phase complex-forming equilibria. A retention model, based on chromatographic separation by an anion-exchange mechanism of metal ions present in the eluent solution as negatively charged complexes, was described by Hajos et al. [2]. Of the above two exchange mechanisms, cation exchange seems to present a wider field of application in the study of association equilibria, starting with positively charged and/or neutral complexes. On the other hand, success in the determination of stability constants via cation-

exchange chromatography depends on the following conditions: (1) the concentration of the complex-forming agents may be varied in as wide a range as possible at constant concentration of the counter ion (competing cation) E^{n+} and constant ionic strength I; (2) this variation must have no effect on the characteristics of the chromatographic column mainly in terms of its capacity Q and its path length; (3) the solute elution is controlled by ion-exchange mechanisms only, regardless of the actual concentration of the complex-forming agent; and (4) departures from the above conditions must meet experimental evidence.

As far as we know, no mention has been made in the literature about any side-effects on the determination of association constants by ion chromatography (IC), most of the papers published on this topic being concerned with mea-

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surements in a short range of concentrations of ligand, C_L, at $I < 0.1$. In these cases, only the first equilibrium step was predominant and data were treated for β_1 calculation only [3–6], sometimes according to a semi-quantitative approach.

The aim of this paper is to show that (i) the nature of both the counter ion and of the co-ion (anion) may affect under some experimental conditions the elution mechanism as a result of one or more of the first three conditions above not being fulfilled and (ii) once some preliminary proof has been obtained for the system of interest, these conditions can be experimentally highlighted so that accurate values of β_i can be obtained, for well defined eluent electrolyte compositions, thus avoiding misleading contributions.

Systematic errors were also considered, which may affect the experimental determination of the capacity factor k' and, in turn, the calculated β_i values, as a function of the concentration C_L in the range from zero up to the maximum allowable value.

The potential of IC in the determination of reliable β_i values, and its peculiar feature of giving auto-consistent information on mileading side mechanisms, were tested for the systems Cd(II)–Cl⁻ and Cd(II)–NO₃⁻, which are used as examples.

2. Theoretical

2.1. Cation-exchange mechanisms in the absence or presence of ligands

For the sake of clarity, the most significant equations related to the above mechanisms are reported here with some comments. Detailed information on this topic is available in the literature [7]. Throughout this paper the analyte cation will be considered as being bivalent and the counter ion monovalent.

In the absence of ligands, the chromatographic exchange process involves the free hydrated species M^{2+} and the counter ion E^- of the eluent, according to

$$M_m^{2+} + 2E_r^+ = M_r^{2+} + 2E_m^+ \tag{1}$$
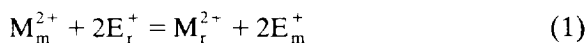
$$K_{M,E} = \frac{[M^{2+}]_r[E^+]_m^2}{[M^{2+}]_m[E^+]_r^2} \cdot \left(\frac{\gamma_M}{\gamma_E^2}\right)_r \left(\frac{\gamma_E^2}{\gamma_M}\right)_m \tag{2}$$

where the subscripts r and m refer to the stationary and mobile phases, respectively, and the other terms have the usual meanings. It is implicitly assumed that the nature of the co-ions of the eluent does not affect the exchange equilibria. This assumption becomes increasingly valid the more the surface of the stationary phase is only occupied by the negatively charged groups, so that adsorption of electrolyte co-ions as the primary step on the resin bed is electrostatically hindered. This is not the case, for example, in gel chromatography, where the distribution of ionic species is taken to be a combined effect of size exclusion and partition, and where the partitioning depends very much on the type of both the counter ion and its co-ion [8].

Apart from the extreme case above, once the distribution and capacity factor have been defined by Eqs. 3 and 4, the experimentally measurable capacity factor depends, in the absence of ligands, on the characteristics of the column and on the eluent according to Eq. 5.

$$D_{M,E} = \frac{[M^{2+}]_r}{[M^{2+}]_m} \tag{3}$$

$$k'_s = D_{M,E} \cdot \frac{V_r}{V_m} \tag{4}$$

$$k'_s = K_{M,E} \cdot \frac{V_r}{V_m} \left(\frac{[E^+]_r}{[E^+]_m}\right)^2 \left(\frac{\gamma_E^2}{\gamma_M}\right)_r \left(\frac{\gamma_M}{\gamma_E^2}\right)_m$$
$$= \frac{V_R - V_0}{V_0} \tag{5}$$

where V_R is the solute retention volume and V_0 is the void volume $(V_0 = V_m)$.

According to Eq. 5, k'_s depends on both I and $[E^+]$. It will be shown later that, in pure cation exchange, the activity coefficient ratio in Eq. 5 may be considered as being independent of I. If a ligand is present in the eluent, Eq. 5 takes the form

$$k_L'(I, [E^+]_m, [L^-]) = \alpha(I, [L^-])k_S'([E^+]_m) \quad (6)$$

where, in addition to the dependence on counter ions, k_L' depends on I, $[L]_m$ and α:

$$\alpha = \frac{[M^{2+}]}{C_M} = \frac{1}{1 + \beta_1[L^-] + \beta_2[L^-]^2 + \cdots} \quad (7)$$

For a given column, at a constant concentration of E^+ and constant I, Eq. 6 becomes

$$\frac{1}{(k_L')_{I,[E]}} = \frac{1}{(k_S')_{[E]}} \cdot (1 + \beta_1[L^-] + \beta_2[L^-]^2 + \cdots) \quad (8)$$

Eq. 8 shows that the intercept of the $1/k_L'$ vs. $[L^-]$ plot gives the value of the term $1/k_S'$. Otherwise, as the k_S' value can also be experimentally obtained for $\alpha = 1$, Eq. 8 can be rewritten as

$$\left(\frac{k_S'}{k_L'}\right)_{I,[E]} = 1 + \beta_1[L^-] + \beta_2[L^-]^2 + \cdots \quad (9)$$

If both the theoretical and experimental conditions hold, Eqs. 8 and 9 show that the capacity factor, obtained at various ligand concentrations and constant I and $[E^+]$, is related to the β_i values of the system without any non-specific contribution. This is a distinguishing feature of IC, for example, compared with potentiometry and calorimetry. In the first case allowance must be made for the non-specific contribution of junction potential on ΔE and in the second case for the non-specific contribution of mixing or dilution heat on ΔQ.

If the metal-containing species (e.g., ML^+ and ML_2) undergo an exchange with the stationary phase, Eq. 6 becomes

$$k_{mix}' = \alpha_{M^{2+}} k_{M^{2+}}' + \alpha_{ML} \cdot k_{ML}' + \alpha_{ML_2} k_{ML_2}' \quad (10)$$

where k_{mix}', the experimental capacity factor, is the sum of the various capacity factors concerning each metal-containing species multiplied by the respective ionic fraction. In terms of β_i and of the ligand concentration:

$$k_{mix}' = \frac{k_{M^{2-}}' + \beta_1[L^-]k_{ML^+}' + \beta_2[L^-]^2 k_{ML_2}'}{1 + \beta_1[L^-] + \beta_2[L^-]^2} \quad (11)$$

Eq. 11 is non-linear in both the β_i and the k_{ML_i}' parameters. In principle, working at constant ionic strength and variable ligand concentrations, fitting of the data will improve by increasing the number of parameters regardless of their physical meaning.

To ascertain the validity of the model assumed, sets of data obtained at different ionic strengths and various concentrations of L are required. Only when all the values of k_{ML_i}', multiplied by the actual counter-ion concentration $(k_{ML_i}'[E^+]^{2-i})$, are found be the same is the validity of the model confirmed. If not, one or more of the k_{ML_i}' values initially assumed must be set to zero and the data reprocessed until consistent values of k_{ML_i}' are obtained regardless of I.

Eq. 11 has been derived only on thermodynamic grounds; however, steric effects may play a very important role in the exchange process. The higher the charge and the larger the size of the complex size, the more important these effects are.

It will be shown later that in the Cd^{2+}–Cl^- system, only the Cd^{2+} species undergoes exchange.

3. Experimental

3.1. Equipment

Two Dionex Series 4000i high-pressure pumps, totally computer controlled, were used for pumping the eluent and the postcolumn derivatization solution. A Dionex UV spectrophotometer was used in both V_0 and V_R detection. The eluent was mixed with the postcolumn derivatization solution in a three-way tee and the mixture was passed to the detector through a bead-packed reaction coil (the total postcolumn dead volume was 450 μl). Eluents, column and postcolumn derivatization systems were thermostated at $25 \pm 0.1°C$.

3.2. Columns

The following Dionex columns were used: a 250×4 mm I.D. and a 50×4 mm I.D. Omnipac PCX 100 IC-cation analytical column, manufactured using microporous substrate beads functionalized with sulphonic groups as cation-exchange sites (surface area 1 m²/g, pore size 60 Å, capacity 120 μequiv. per column); a 250×4 mm I.D. Omnipac PCX-500 IC-cation analytical column, manufactured using macroporous substrate beads functionalized with sulphonic groups as cation-exchange sites (surface area 300 m²/g, pore size 60 Å, capacity 120 μequiv. per column) was also used in some preliminary experiments.

3.3. Chemicals

Carlo Erba RPE doubly distilled water, Baker Instra-Analyzed perchloric acid, Merck Suprapur hydrochloric acid, Baker Analyzed sodium chloride, Baker Analyzed sodium perchlorate monohydrate and Baker Analyzed acetonitrile were used in the eluent preparation. Aldrich 2-(5-bromo-2-pyridylazo)-5-diethylamino)phenol, Baker Analyzed Triton X-100, Baker Analyzed boric acid and Baker Analyzed sodium hydroxide were used for the derivatization solution.

3.4. Eluents

For each ionic strength (0.05, 0.10, 0.22, 0.30, 0.50), stock standard solutions of $HClO_4$ and HCl were prepared from the corresponding commercial concentrated solutions. The correct molarities of the stock standard solutions were determined by titration with Na_2CO_3. In addition, stock standard solutions of $NaClO_4$ and NaCl, for each ionic strength, were prepared from the corresponding sodium salts; these stock standard solutions contained 0.01 M H^+. Working standard solutions were prepared by diluting appropriate volumes of the stock standard solutions. All the eluents contained 1% of acetonitrile to ensure that the column packing was wetted properly.

3.5. Procedures

In all experiments concerning cadmium elution, 25 μl of a 5 ppm Cd in 0.01 in 0.01 M HNO_3 solution were injected. Cd detection was performed by using a postcolumn derivatization system: a solution of 2-(5-bromo-2-pyridylazo)-5-diethylamino)phenol (0.4 mmol at pH 10, adjusted with 0.5 M sodium borate–boric acid buffer) was mixed with the eluent at the column end (the UV detector was set at 565 nm).

When using the postcolumn derivatization device, the mobile phase volume of the column (V_0) was determined at 300 nm from the positive peak of NO_3^-; otherwise, V_0 was determined by detecting the peak elution of nitrate at 230 nm and/or the negative elution peak of the solvent at 205 nm.

A flow-rate of 1 ml/min was used for all the eluent compositions with the exception of $I = 0.5$, where 0.5 ml/min was used to improve the measurements of both T_R and T_0. A flow-rate of 0.2 ml/min was used for the derivatization solution.

4. Results and discussion

4.1. Experimental evidence (in perchloric acid medium) for the type of dependence of the activity coefficient ratio on the ionic strength and on the type of chromatographic column

Eq. 2 predicts that when the concentration of a uni–univalent electrolyte, containing E^+ as a counter ion, is changed, the product $k'[E^+]^n$ will vary as the activity coefficient ratio, R, does:

$$k'[H^+]_m^n = a\left(\frac{\gamma_H^n}{\gamma_M}\right)_r\left(\frac{\gamma_M}{\gamma_H^n}\right)_m = aR \qquad (12)$$

where n is the charge of the solute ($n = 2$ in our example) and $a = K_{ME}(V_r/V_m)[H^+]_r^n$. Expressed in logarithmic form, Eq. 12 becomes

$$\log(k'[H^+]_m^n) = \log a + \log R \qquad (13)$$

Haddad and Foley [1] reported the slopes of log k' vs. log(mean activity of perchloric acid)

for bi- and trivalent solutes as obtained according to Refs. [9,10]. Depending on the nature of the solute, the relevant value of the slope was found to vary from the theoretical value $(-n)$, sometimes significantly. For instance, the experimental values of the slope were -1.66 for Mg^{2+}, -1.87 for Ni^{2+} and -2.08 for Cd^{2+}, instead of -2.

Apart from the questionable use of the activity of perchloric acid instead of I (the activity coefficient of the counter ion H^+ is included in R), these behaviours appeared to be due more to different partition mechanisms than to a different (and even counter) effect of the solutes on the activity coefficient ratio. Specifically, depending on the column features, we expected that the adsorption of solute and counter ions on the resin bed might give rise to an inverse phase elution mechanism, in parallel with the exchange mechanism. Since the specific adsorption of both solute and counter ions was also considered by Stáhlberg [11] in his double-layer model, we decided to carry out additional experiments whereby values of k'_s for the above solutes Cd^{2+} and Ni^{2+} were obtained, using different columns, both at various counter-ion concentrations and I (perchloric acid). If experimental conditions had been found where R does not depend (or only very slightly) on I, one could confidently expect, when studying association equilibria, that changes in the nature of the salt composition in the eluent, at constant I, would be made without significant misleading effects. It was found that:

(i) When using a micropore substrate bed column (surface area 1 m^2/g) the slope of $\log aR$ vs. $\log I$ plot can be considered constant and near to zero in the range of I used (0.06–0.5): the maximum variation in aR between the limit concentrations of $HClO_4$ was 4% and 7% for Cd^{2+} and Ni^{2+}, respectively (see Fig. 1, curves a and b); the shape of the chromatograms for both Cd^{2+} and Ni^{2+} is regular regardless of I.

(ii) When using a macropore substrate bed column (surface area 300 m^2/g) with the same fixed charge equivalents Q as before, the dependence of $\log aR$ on $\log I$ is clearly outside the experimental uncertainty (see Fig. 1, curve c), while the shape of the chromatogram is distorted
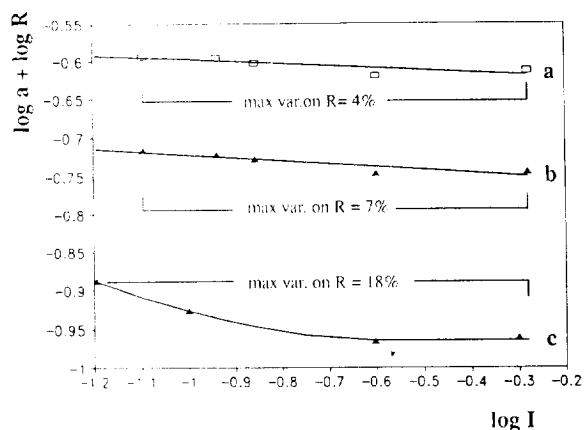

Fig. 1. Effect of ionic strength ($HClO_4$ medium) on the activity coefficient ratio (see Eq. 12). Solutes considered: Cd (curve a) and Ni (curves b and c). Curves a and b refer to a micropore column (Dionex PCX100) and curve c to a macropore column (Dionex PCX500).

as shown in Fig. 2b for Ni^{2+}: the lower the $HClO_4$ concentration, the greater is the distortion.

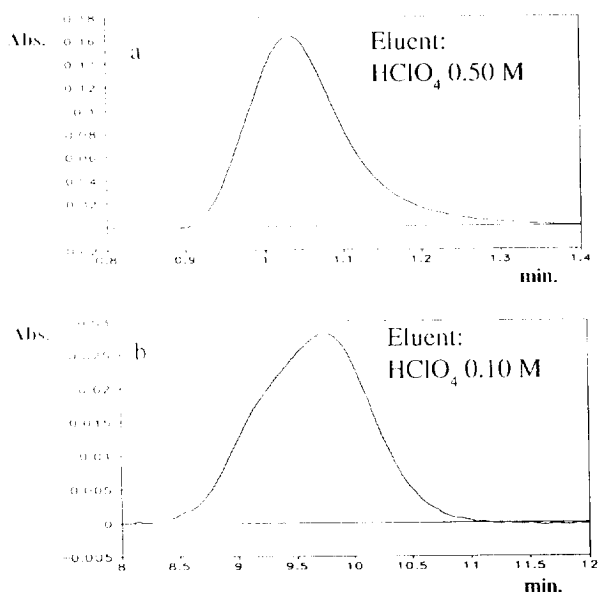The above experiments, performed at various

Fig. 2. Evidence of parallel mechanisms of elution from the dependence of the shape of the chromatogram (Ni^{2+}) on the counter-ion concentration at variable I ($HClO_4$ medium). Dionex PCX500 macropore column.

I (HClO$_4$ medium) and in the absence of ligands, show that parallel mechanisms of elution are possible. They should be minimized by using columns where only the ionic functional groups are active at the surface of the stationary phase, as the free sites on the surface of the resin bed, if any, are preferentially occupied by protons so that the elution of solute is controlled by the ion-exchange mechanism. Under these conditions, the activity coefficient ratios of the species involved in the exchange equilibrium are fairly independent of I.

4.2. Effects of the nature of the counter ion on the Cd^{2+} capacity factor

Once it had been ascertained that hydrogen ions may hinder the specific adsorption of solute ions such as Cd^{2+} and Ni^{2+} on the surface of the resin bed, other monovalent species were tested, such as sodium, to verify whether they play the same role as H$^+$ and under what experimental conditions. This is an important issue whenever the ligand to be considered in equilibrium studies is a weak acid or base, so that pH is not an independent variable.

These experiments were performed using the micropore resin bed column, Cd^{2+} as a solute and a mixture of HClO$_4$ and NaClO$_4$ at constant I (0.1 or 0.3) in the eluent. It was found that:

(i) At $I = 0.1$ the equivalent fraction of H$^+$ (N_{H^+}) added to the mixture can be lowered to zero (pH 7); the shape of k' vs. N_{H^+} plot assumes the form shown in Fig. 3, which is different from that predicted by the replacement of H$^+$ with Na$^+$ as a counter ion according to the equation

$$k'_s = \frac{V_r}{V_m} \cdot Q^2 K_{M,H} \left([H^+] + [Na^+]\sqrt{\frac{K_{M,H}}{K_{M,Na}}} \right)^{-2}$$
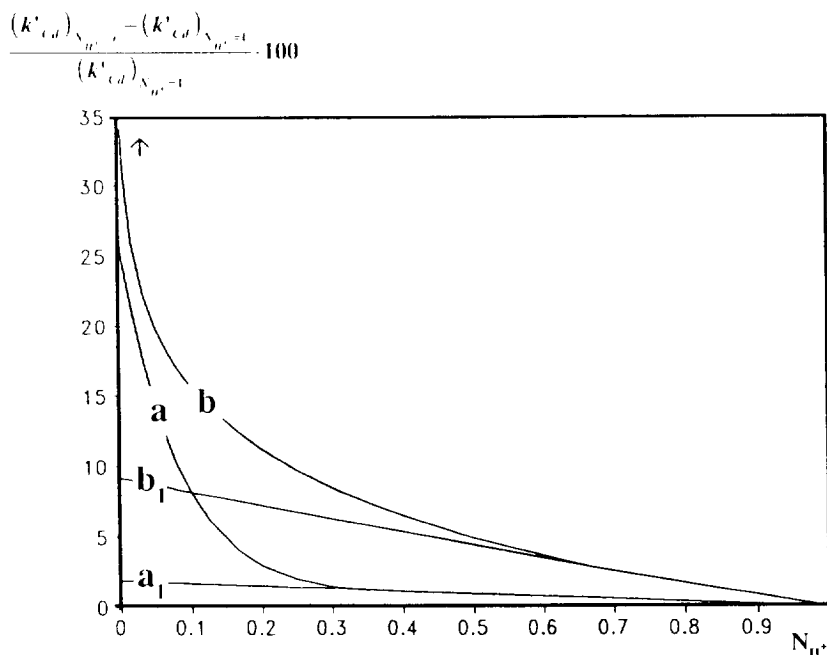
$$(14)$$

where the term $(V_r/V_m)Q^2 K_{M,H}$ can be obtained

Fig. 3. Effect of the nature of the counter ion (Na$^+$ or H$^+$) on Cd(II) capacity factor in perchlorate medium at constant ionic strength. I = (a) 0.10 and (b) 0.30; curves a$_1$ and b$_1$ refer to the theoretical shapes calculated at the respective ionic strength according to Eq. 14.

from the experimental values of k'_s at $N_{H^-} = 1$, and $K_{M,Na}$ is an unknown parameter. The $K_{M,Na}$ value can be estimated by using the k'_s values in the N_{H^+} region where k' is a nearly linear function of N_{H^+}.

(ii) At $I = 0.3$, a minimum value of $N_{H^+} = 1.7 \cdot 10^{-3}$ ($C_{H^-} = 5 \cdot 10^{-4}$ M) is required to avoid irreversible adsorption of Cd^{2+}.

4.3. Effect of the nature of the co-ion in the eluent

In cation-exchange chromatography, the nature of the anions present in the eluent should have no effect on the performance of the chromatographic column, at least when the functional fixed charges are close together [8,12]. It will be shown that, when measuring V_0 by using nitrate solute, the plot of V_0 vs. N_{Cl} shows a slope variation in the region of N_{Cl^-} 0.9–1. In the same interval the $k'_{Cd^{2+}}$ vs. N_{Cl} plot shows anomalous behaviour. Table 1 summarizes the results obtained by using both the solvent and nitrate peaks in measuring V_0. Each value is the mean of nine replicates; the sample relative

Table 1
Measurements of V_0 at various ionic strengths (Dionex PCX100 microporous substrate bed column)

Ionic strength	Eluent	Peak elution considered		Δ
		H_2O	NO_3	
1.00	HCl	1.105	1.114	−0.009
	$HClO_4$	1.110	1.085	0.025
	Δ	−0.005	0.029	
0.50	HCl	1.106	1.136	−0.030
	$HClO_4$	1.127	1.102	0.025
	Δ	−0.021	0.034	
0.30	HCl	1.123	1.145	−0.022
	$HClO_4$	1.125	1.100	0.025
	Δ	−0.002	0.045	
0.10	HCl	1.096	1.158	−0.062
	$HClO_4$	1.104	1.084	0.020
	Δ	−0.008	0.074	
0.05	HCl	1.076	1.193	−0.117
	$HClO_4$	1.080	1.062	0.018
	Δ	−0.004	0.131	

standard deviation was typically 0.1% and the repeatability of mean V_0 among days was 0.2% so that differences between V_0 mean values higher than 0.1% ($\alpha = 0.5$) were statistically highly significant. It was found that:

(i) With $HClO_4$ solutions, V_0 decreases when the acid concentration is increased from 0.05 to 0.5 M, regardless of the criteria used to measure it. The variation in V_0, ΔV, using the solvent and nitrate peaks, was found to be positive and increased from 0.05 M (0.018 ml) to 0.3 M (0.025 ml).

(ii) With HCl solutions, V_0 increases up to 0.3 M when the solvent peak is used, and continuously decreases with decreasing acid concentration when the NO_{3^-} peak is used. The greatest variation between the V_0 values obtained by the two methods of measurements was found at 0.05 M ($\Delta V = 0.118$) and the smallest at 1 M ($\Delta V = 0.009$).

As shown in Fig. 4, where V_0 and k'_s/k'_L vs. N_{Cl^-} plots are compared, both V_0 [measured in the absence of Cd(II)] and k'_s/k'_L for Cd(II) present consistent variations in slope within almost the same N_{Cl^-} interval. Thus, whenever V_0 vs. N_{X^-} presents a shape comparable to that shown in Fig. 4 for Cl^-, sound information is obtained about the useful range of N_{X^-} in measuring equilibrium constants.

From all the above experimental findings, including those on the effect of the nature of the counter ion on the capacity factor, a model can
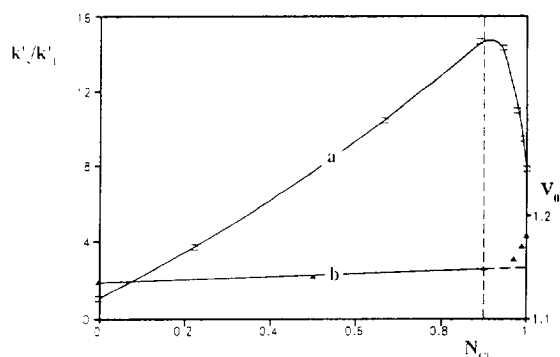

Fig. 4. Effect of substitution of ClO_4^- by Cl^-: (a) on k'_s/k'_L and (b) on V_0 (nitrate as solute). Cd^{2+}–Cl^- system, $I = 0.50$ M. Dionex PCX100 microporous column.

be proposed, however rough, to interpret and correlate the data, as follows.

(i) The resin matrix cannot be exactly considered as a plane sheet to which a surface area corresponds. Pores or some surface points, where functional groups are lacking, are possible depending on the resin's peculiar characteristics, i.e., area per gram or equivalents per square metre. Whenever any interaction by the reversed-phase mode of solute with pores or with free surface points is (or is made) not significant, all the assumptions concerning the surface complexation model [10,13,14] hold. In particular, the electrical charge of the fixed sites is neutralized by the different types of counter ions which are arranged in characteristic individual Stern layers [11]. The Stern layers contain most of the counter ions; further counter ions are distributed in the adjacent layer with a diffuse distribution of counter and co-ions.

(ii) In micropore substrate resins, whenever the number of resin equivalents per unit area is very large and charges are very close, direct specific adsorption (on free resin bed sites) of anions as a primary step is hindered, while some cation adsorption is still possible, owing to the synergic effects of the electrostatic field due to the negative fixed charges, and the hydrophilic-like bed surface–solute interaction. Once the cation has been specifically adsorbed, the anionic co-ion can be bound on the same surface plane forming ion pairs, or arranged in an outer layer, inside the double layer. It is likely that relatively hydrophobic co-ions, such as perchlorate, may enhance and stabilize the direct cation adsorption. In principle, the charges of the specifically adsorbed cation can be in part neutralized by the negative charges of the functional groups.

(iii) When pores and free surface sites are effective in promoting solute-specific adsorption on a micropore porous resin surface, they affect the solute elution mechanism the greater is the equivalent free surface area on the resin bed and the weaker is the competitivity of counter ions of the eluent in counteracting the solute adsorption. This may explain the elution behaviour of Cd ion in perchlorate medium, at variable equivalent fraction of protons and sodium ion, found at two

different I (0.1 and 0.3). When N_{H^+} goes below 0.3 ($I = 0.1$) or 0.7 ($I = 0.3$) to zero (see Fig. 3, curves a and b, respectively), the cadmium elution increasingly departs from the cation-exchange mechanism as its adsorption competes with the proton or sodium adsorption at their respective concentrations in the eluent. Specifically, at $I = 0.3$ the antagonistic effect of sodium alone in reducing cadmium adsorption is no longer sufficient (salting-out effect?) with N_{H^+} decreasing to zero. A finite concentration of protons is required to avoid complete irreversible adsorption of cadmium ions (see Fig. 3, curve b).

When the microporous substrate is replaced by a macroporous substrate, owing to the very large increase in the surface area, sites are possible where the charge effect of the functional groups is too weak to repel surface adsorption of anions. As a second step, ion-pair formation with multicharged cations is favoured. This accounts for the need to use very high concentrations of protons (>0.1 M) to counteract Cd ion adsorption, when macropore resins are used.

(iv) In an eluent containing ClO_4^- and Cl^- at constant I, when the equivalent fraction of Cl^- becomes higher than 0.8, the specific adsorption of the $H^+ClO_4^-$ ion pairs decreases. This decrease is in part compensated by the formation of ion pairs between protons and chloride ions. Unlike ClO_4^-, chloride ions are not specifically adsorbed as co-ions but are located in the double layer. The amount of Cl^- sorbed decreases, at constant N_{Cl^-}, as I increases (because of the desorption of the univalent cation). When Cl^- is sorbed in the double layer, the resin acts as both a cation and anion exchanger. This infers that, at increasing N_{Cl^-}, V_0 will present a higher variation the lower is I and k'_s/k'_L a lower variation the lower is I.

The equivalent capacity of the anion exchanger depends on the amounts of sorbed Cl^-, that is, it depends on N_{Cl^-} and I. This accounts for the following findings: when nitrate is used as a solute in measuring V_0 at different N_{Cl^-} values and these values are compared with that obtained at $N_{Cl^-} = 0$ at the same ionic strength, the relevant ΔV is always higher than zero in all the

situations considered because the capacity factor of nitrate is no longer zero under these conditions, but tends to zero as N_{Cl} decreases or I increases; when Cd^{2+} is used as a solute, since the proton specific adsorption (which is much larger than sodium ion adsorption) decreases as N_{Cl^-} or I increases, the reversed-phase mode exchange becomes more significant in the elution of Cd^{2+} the higher is N_{Cl} or I, with a consequent anomalous increase in the observed capacity factor k'_{Cd} (Fig. 4). All the above considerations are based on the stoichiometric model for data interpretation, the only one that is available as far as we know for solutes distributed among several different metal-containing forms. Further, the electrostatic retention model in developing the surface complex formation with each counter-ion species arranged in the double layer specifically excludes the presence of free sites, so differences in behaviour between macro- and microporous resin beds cannot be explained.

It is interesting to compare our findings on the dependence of the cadmium capacity factor on N_H, at constant I, with those of Günter, discussed by Höll et al. [14,15] on the distribution of calcium ion and protons on a strong acid resins. Günter's experiments were performed under true equilibrium conditions by pouring increasing amounts of resin, in the calcium form, into a hydrochloric acid solution of a given concentration, and after some days measuring the concentrations of the solutes in both phases. It was found that for small resin loadings, the proton concentration in the resin phase is higher than expected with a consequent desorption of some bivalent ions. This agrees with our findings about preferential adsorption of protons with respect to cadmium ion.

4.4. Conclusions about preliminary tests in IC investigations of complex equilibria

In order to minimize the effects due to parallel mechanisms of elution (reversed-phase interaction), from the above findings the following conclusions and recommendations are proposed:

(1) In the stationary phase the number of equivalents of fixed groups per square metre has to be as high as possible.

(2) At constant total concentrations of competing cations, a minimum concentration of hydrogen ion is suggested in the eluent, whenever possible, to prevent adsorption phenomena on the stationary phase bed. If not, the value of k'_s, at zero ligand concentration, may be affected by error, the higher is the ionic strength.

(3) When the ligand is anionic and the ionic strength is kept constant with perchlorate, the maximum ligand concentration in the eluent must not reach 100% of the total anionic concentration.

Point (1) can be verified by plotting log $k'_s[E^-]^n$ vs. I and from the shape of the solute chromatographic curves. Point (2) can be verified from the plot of k'_s vs. $[H^+]$ with $[H^+]+[E^-] = $ constant and $[H^+]$ varying from 0 to 100%. Point (3) can be verified from the plot of k'_s/k'_L vs. $[A^-]$ (or $[ClO_4^-]$) at constant total concentration of counter ions at varying $[ClO_4^-]$ and/or from the plot of V_0 vs. $[L^-]$ at $[L^-]+[ClO_4^-] = $ constant.

4.5. Cadmium–chloride and cadmium–nitrate ionic complexes

Once the experimental conditions for the cadmium–chloride system had been defined, whereby the chromatographic ion elution is purely controlled by the ion-exchange mechanism, measurements were made at 0.50, 0.30, 0.22, 0.10, and 0.05I. The ionic fraction of ligand was varied from 0 to 0.8. When a mixture of sodium and hydrogen ions in the eluent was used, the concentration of proton was kept constant and equal to 0.01 equiv./l. Table 2 shows the most significant values. The thermodynamic $_T\beta_i$ values were roughly estimated by using the activity coefficient values calculated by Kielland [23] at the operating ionic strength (0.05, 0.10). The agreement with literature data is fairly good. The same holds for β_1 at $I = 0.1$ and 0.5, for which literature data are available. For β_2 at $I = 0.5$, good agreement was found

Table 2
Comparison of IC results with literature data for the Cd(II)–Cl system

I	$_T\beta_1$	β_1	β_2	Method[a]	Ref.
0	$95.5 \pm 7.2\%$				[16]
0	93.54			Pot.	[17]
0	93.5			Pot.	[18]
0.05[b]	97.0[d]	44.3		IC	TW[e]
0.05[b]	92.9[d]	42.4		IC	TW[e]
0.10		$38.9 \pm 9.6\%$		Kin.	[19]
0.10[b]	95.4[d]	$35.3 \pm 1.4\%$	$77.4 \pm 8.5\%$	IC	TW[e]
0.22[b]		$27.8 \pm 1.3\%$	$66.5 \pm 4.1\%$	IC	TW[e]
0.22[b]		$27.6 \pm 1.2\%$	$61.1 \pm 3.9\%$	IC	TW[e]
0.30[b]		$26.1 \pm 1.0\%$	$46.0 \pm 1.3\%$	IC	TW[e]
0.30[c]		$26.1 \pm 0.3\%$	$40.7 \pm 0.6\%$	IC	TW[e]
0.50[c]		$23.4 \pm 1.5\%$	$34.2 \pm 4.2\%$	IC	TW[e]
0.50[c]		$21.9 \pm 0.5\%$	$33.8 \pm 6.1\%$	IC	TW[e]
0.5		22 ± 4	33 ± 6	Pot.	[20]
0.5		23.5	57	Pot.	[21]
0.5		23.5 ± 0.2	63 ± 2	Cal.	[22]
0.5		$22.39 \pm 4.7\%$	$50.12 \pm 26\%$		[14]

[a] Pot. = potentiometry; IC = ion chromatography; Kin. = kinetic; Cal. = calorimetry.
[b] The eluent composition is a proper mixture of solutions 1 and 2 at the given ionic strength (I): (1) HCl; (2) $HClO_4$.
[c] The eluent composition is a proper mixture of solutions 1 and 2: (1) NaCl ($I - 0.01\ M$) + $HClO_4$ ($0.01\ M$); (2) $NaClO_4$ ($I - 0.01\ M$) + $HClO_4$ ($0.01\ M$).
[d] As a rough estimate, assuming that the activity of coefficient values of Kielland [23] at the operating I are correct.
[e] This work. These data include the confidence interval percentage ($\alpha = 0.05$); data from literature include the percentage of uncertainty.

with the data from Ref. [18]. Note that the β_2 data from Refs. [18] and [19], which differ by a factor of about two, were both obtained from potentiometric measurements using a transport-type cell. In the former case a calomel electrode was used in $LiCl–LiClO_4$ medium and in the latter case a cadmium amalgam in $HClO_4–NaClO_4$ medium was used as a reference electrode; clearly, the wide difference in β_2 values arises, at least in one case, from ineffective elimination of the junction potential contribution. The relative confidence interval at the 0.05 significance level and the repeatability among different sets of data are particularly good. This is confirmed by the data relating to the cadmium–nitrate complexes. In this case, as the stability constant is low ($\beta_1 = 0.60$ at $I = 0.5$) and the set of k'_s/k'_L data are in the range 1–1.15, one would expect a wider relative confidence interval for β_1 than in chloride; in fact, as Table 3 shows, the confidence interval found was 2%. This is

further proof that very low stability constants can be accurately measured by IC without non-specific contributions.

Table 3
Comparison of IC results with literature data for the Cd(II)–NO_3^- system

I	β_1	Method[a]	Ref.
0.05	0.31	Pot.	24
0.10	$1.56 \pm 2.0\%$	IC	TW[b]
0.1	$1.86 \pm 14.8\%$	Kin.	17
0.1	1.25	Extraction	25
0.30	$0.78 \pm 1.2\%$	IC	TW[b]
0.45	$0.62 \pm 1.5\%$	IC	TW[b]
0.5	$0.60 \pm 1.6\%$	IC	TW[b]
0.5	0.77 ± 0.02	Pot.	18
0.5	0.776		14
1	$1.17 \pm 17.5\%$	Kin.	24

[a] See Table 2.
[b] This work.

4.6. Sources and sizes of systematic errors affecting calculated k' values

The accuracy of k' calculated from chromatographic experiments depends both on the accuracy of all the variables considered in Eq. 6 and on the accuracy of the V_R and V_0 measurement from the chromatogram:

$$k' = \frac{V_R - V_0}{V_0} = \frac{T_R - T_0}{T_0} \tag{15}$$

From Eqs. 6 and 15, it is possible to obtain an equation for the error affecting the capacity factor k', expressed as $\Delta k'$.

The $\Delta k'$ dependence on all the variables is expressed by the following equation:

$$\Delta k' = \frac{\delta k'}{\delta V_R} \cdot \Delta V_R + \frac{\delta k'}{\delta V_0} \cdot \Delta V_0 + \frac{\delta k'}{\delta [L^-]} \cdot \Delta [L^-]$$
$$+ \frac{\delta k'}{\delta [E^-]} \cdot \Delta [E^-] \tag{16}$$

Considering the partial derivatives for the different terms in Eq. 16:

$$\frac{\delta k'}{\delta V_R} = \frac{1}{V_0}; \quad \frac{\delta k'}{\delta V_0} = -\frac{V_r}{V_0^2};$$

$$\frac{\delta k'}{\delta [L^-]} = -\frac{k_s'(\beta_1 + 2\beta_2[L^-])}{(1 + \beta_1[L^-] + \beta_2[L^-]^2)^2}; \tag{17}$$

$$\frac{\delta k'}{\delta [E^+]} = -2 \cdot \text{constant} \cdot \frac{1}{[E^+]^3}$$

one has for the relative bias

$$\frac{\Delta k'}{k'} = \frac{V_R}{V_R - V_0}\left(\frac{\Delta V_R}{V_R}\right) - \frac{V_R}{V_R - V_0}\left(\frac{\Delta V_0}{V_0}\right)$$
$$- \frac{\beta_1 + 2\beta_2[L^-]}{1 + \beta_1[L^-] + \beta_2[L^-]^2} \cdot \Delta[L^-]$$
$$- 2 \cdot \frac{\Delta[E^-]}{[E^-]} \tag{18}$$

As we found experimental evidence that the flow-rate of the eluent v' does not change during a chromatographic run, the value of k' calculated by Eq. 15 is independent of the flow-rate reproducibility while the values of V_R and V_0 (or T_R and T_0) may change among days. Assuming for $|\Delta V_R|$ and $|\Delta V_0|$ an uncertainty equal to
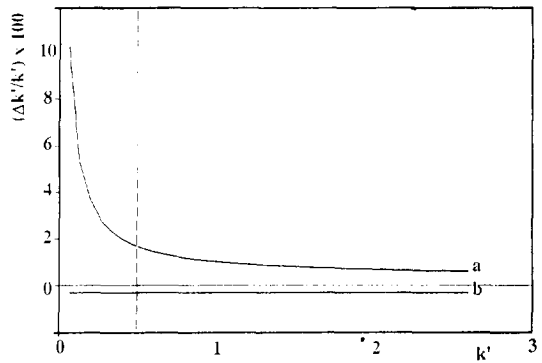

Fig. 5. Effect of errors in V_R and V_0 estimation on k' values. $|\Delta V_R| = |\Delta V_0| = 5 \cdot 10^{-3}$ ml from experimental evidence: (a) $\Delta V_R = -\Delta V_0$; (b) $\Delta V_R = \Delta V_0$.

$5 \cdot 10^{-3}$ ml for the same set of data when using the postcolumn derivatization device, according to whether they have the same sign or not, the $\Delta k'/k'$ (%) values assume the shape in Fig. 5 (curve a or b). It follows that values of k_L' lower than 0.5 and consequently values of α lower than $0.5/k_s'$ (see Eq. 9) are imprecise.

The other terms that affect the size of $\Delta k'$ are due to systematic errors in the preparation of the individual solutions at constant $[E^+]$ and variable $[L^-]$. Eq. 18 shows that the bias of k' due to systematic error in C_L determination depends not only on the size of ΔC_L but also on the particular association equilibria considered. Further, the ΔC_L and ΔC_E values depend on the different ways in which stock and working standard solutions are prepared; for this reason, no examples are considered here.

5. Conclusions

As has been demonstrated for the cadmium–chloride and cadmium–nitrate systems, accurate values of equilibrium constants can be achieved by cation-exchange chromatography, provided that non-specific contributions on the capacity factor of the solute are nullified. These non-specific contributions may originate from the nature of the competing and or co-ions in the

eluent and/or the presence of free sites in the exchange resin surface. Several preliminary tests have been proposed that enable the correct experimental conditions to be identified with confidence.

Acknowledgements

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