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Histidine as a dipolar eluent component in cation chromatography II. Prediction of retention data for alkaline and alkaline-earth ions

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Abstract

The utility of cation chromatography has been developed by the application of L-histidine as a multiprotic and dipolar (zwitterionic) eluent component. The method simplifies the cation analysis. The chromatographic characteristics of this system were studied in detail with a view to determining the selectivity and the mechanism by which the cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) are retained. Complete separations were observed in the isocratic run over the eluent concentration range 3.0–6.0 m*M* at pH below 2.0. Sensitive detection was achieved using suppressed conductivity at the pH of isoelectric point of the histidine. Retention equations are derived for mono- and divalent cations eluted from ion-exchange separation column with multiple ionic eluents. The theory is based on the extension of ion-exchange equilibrium by protonation equilibria. The selectivity data for analyte and eluent species are determined using the model from the experimental retention data by computer-assisted iterative calculations. The model was utilized to predict retention data. The results in three-dimensional retention surfaces together with species distribution graphs are presented.

Keywords: Mathematical modelling; Retention models; Mobile phase composition; Retention prediction; Inorganic cations; Histidine

1. Introduction

In ion chromatography, the eluent composition provides the main flexibility for manipulating the retention and detection of solute ions. The eluent components used in ion chromatography with suppressed conductivity detection must be such that they are removed from the eluent flow or converted to weakly conducting compounds by the suppressor. The net effect of the suppressor therefore is to reduce the background conductance of the eluent and to enhance simultaneously the detectability of the analyte ions. Selection of an eluent is thus crucial to obtain clean separation and low level detection. The specifications that apply to a substance to be used as eluent in suppressed chromatography are strict. The eluent strength must be reasonably high without poisoning the column. It should not cause additional peaks as a product of the suppressor reaction.

In previous study [1] we have found the acidified L-histidine [2-amino-3-(4-imidazolyl)propionic acid] as a relatively unexplored mobile phase in membrane suppressed cation chromatography. The main advantages of the eluent are its simplicity and detectability since the separation can be performed under isocratic conditions for mono- and divalent cations and the suppressor converts the eluent to its dipolar form, $^+HHis^-$, which has no intrinsic conductivity.

Dipolar eluents aminoalkylsulfonic acid salts [2,3] have been used in suppressed anion chromatography earlier. A micromembrane suppressor combined with

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a mixture of 2,3-diaminopropionic acid and hydrochloric acid is now used [4] for the separation of cations. Efficient suppression by the anion-exchange membrane in aqueous solution is governed mainly by the isoelectric pH (p*I*) of dipolar component. The p*I* value of histidine (7.56) matches this requirement preferably, which is evident from the excellent suppressibility of the eluent and sample cations. Theoretical and practical treatments of separation of cations in liquid chromatography have been reported extensively in the recent literature [5,6].

In our previous paper [1] the retention and detection behaviors of common mono- and divalent cations (Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}) were examined using acidified histidine as eluent. The applicability of the mobile phase was demonstrated by the trace analysis of cations in secondary coolant water of nuclear power plant. We turn now an evaluation of the possible retention mechanism and model for use in cation chromatography. The question of how the chemical composition of an amphoteric eluent affects the selectivity of the system for various cations is an important one. Effective utilization of ion-chromatography requires accurate characterization of analyte retention behavior and identification of analytical variables that affect the relative retention of these analytes. Our retention model [7] has since been amplified and modified [8,9] in the light of new experimental data and it has been used in general optimization strategies [10]. A major advantage of the model is that it takes into consideration all forms of the eluent and analyte ions. The main purpose of the present work is to apply the ion-exchange retention model using a multispecies amphoteric eluent for cation separation and to provide quantitative data on the selectivity of the system.

2. Theory

We will use and test the retention model in two ways. First, the model in the modified form allows the determination of the selectivity coefficients from experimental data. Second, the model have focused on its ability to predict retention data using these constants in terms of derived equations. The theory is based on the extension of ion-exchange equilibrium by protonation equilibria.

The histidine molecule has three sites available for ion-exchange co-ordination. These are an imidazole nitrogen atom, and the amino acid carboxylic and amino groups. For the formation of histidine cations, three equilibrium constants can be considered. These are $K_1=10^{9.10}$, $K_2=10^{6.02}$ and $K_3=10^{1.60}$ [11]

The proton ionisation equilibria of the eluent may be represented:

$$His^- \underset{pH\ 9.10}{\overset{K_1}{\Leftrightarrow}} {}^{+}HHis^- \underset{pH\ 6.02}{\overset{K_2}{\Leftrightarrow}} H_2His^+ \underset{pH\ 1.60}{\overset{K_3}{\Leftrightarrow}} H_3His^{2+} \quad (1)$$

The eluent exists mainly as the divalent cation, H_3His^{2+}, at lower than pH 1.60. It is evident from the protonation equilibria (see Fig. 1) that the elution and suppression behaviour can be easily governed by the pH of the acidified histidine. With p$K=1.60$ and 6.02 for the protonation of the imidazole nitrogen atom and the carboxyl group, histidine may exist in the mobile phase as monovalent, divalent cation or mixture of both, respectively. In any case (pH$>$ 6.02), the results of the suppressor reaction are mainly dipolar or anionic form of the eluent. For this reason, the eluent pH can be considered to be optimized at pH<2 in the separation mode and at pH$\cong$p*I* in the detection mode. As the mobile phase pH is changed simultaneous ion-exchange equilibria and protonation will take place in the chromatographic system.

2.1. Ion-exchange equilibria of sample cations (M^{y+})

The ion-exchange equilibrium for binding of a cation (M) to stationary phase (R) that has been conditioned with eluent (E) is given by:

$$yR_x - E + xM^{y+} \overset{K_{M/E}}{\Leftrightarrow} xR_y - M + yE^{x+} \quad (2)$$

where E^{x+} denotes the H^+, H_2His^+ and H_3His^{2+} ions all act as competing cations in eluent. At equilibrium, the concentrations are interrelated according to the following relation:

$$K_{M/E} = \frac{(M^{y+})^x [E^{x+}]^y}{[M^{y+}]^x (E^{x+})^y} \quad (3)$$

Parentheses indicate the concentrations in the solid

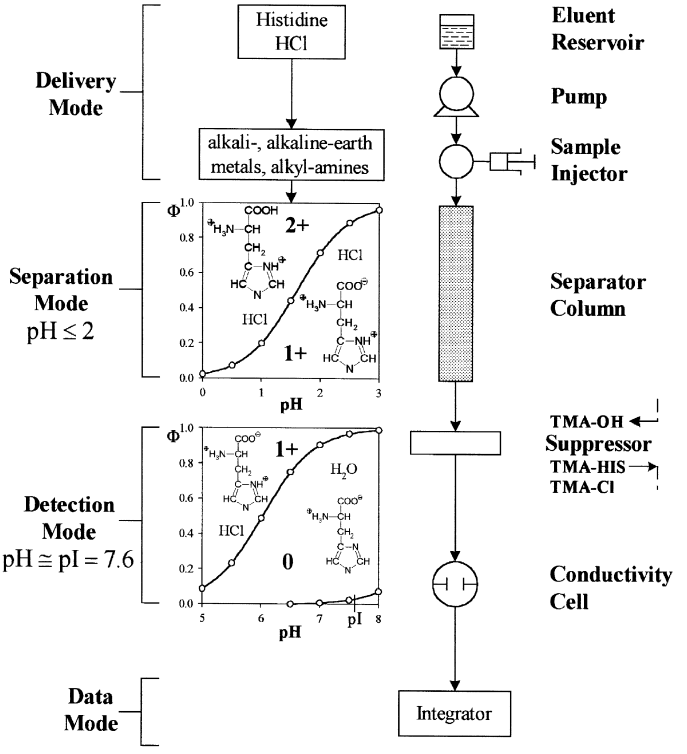

Fig. 1. Schematic flow diagram of system configuration. Species distribution graphs showing the fraction (Φ) of histidine at pH≤2 in the separation mode and at pH≅pI in the detection mode.

and square brackets for the solution phases. We can assume that the eluent occupies all of ion-exchange coordination thus the (E^{x+}) given by the ion-exchange capacity (Q). The volumetric distribution of M^{y+} can be given in terms of $K_{M/E}$ and the ion-exchange capacity of the separator column:

$$D_M = \frac{(M^{y+})}{[M^{y+}]} = K_{M/E}^{1/x} \cdot \left(\frac{(E^{x+})}{[E^{x+}]}\right)^{\frac{y}{x}} = K_{M/E}^{1/x} \cdot \left(\frac{Q}{x}\right)^{\frac{y}{x}} \cdot [E^{x+}]^{-\frac{y}{x}} \quad (4)$$

which may be solved for D_M in terms of the known quantities x, y, Q, $K_{M/E}$ and $[E^{x+}]$. Only a small fraction of all ionic components as monovalent and single competing ions in the elution can be treated by this formalism. It is assumed in this approach that competing eluent species have similar selectivities for solute ions. However, ion-exchange equilibria and retention data are strongly dependent on interactions between the eluent species in the mobile phase.

2.2. Ion-exchange equilibria of eluent ions [E^{x+}]

At the eluent pH values used for separation more eluent components will be present with different elution behavior. In the presence of eluent species (H^+, H_2His^+ and H_3His^{2+}) simultaneous ion-exchange equilibria will take place. Taking H_2His^+ as the basis for eluent ($x=1$) the following equilibria between the competing eluent must be considered:

$$H_3His^{2+} + 2R - H_2His \overset{K_{H_3His^{2+}/H_2His^+}}{\Leftrightarrow} R_2 - H_3His + 2H_2His^+ \quad (5)$$

$$H^+ + R - H_2His \overset{K_{H^+/H_2His^+}}{\Leftrightarrow} R - H + H_2His^+ \quad (6)$$

The intereluent equilibrium coefficients for these processes are:

$$K_{H_3His^{2+}/H_2His^+} = \frac{(H_3His^{2+})[H_2His^+]^2}{[H_3His^{2+}](H_2His^+)^2} \quad (7)$$

$$K_{H^+/H_2His^+} = \frac{(H^+)[H_2His^+]}{[H^+](H_2His^+)} \quad (8)$$

The ion-exchange capacity of the stationary phase is given by:

$$Q = 2(H_3His^{2+}) + (H_2His^+) + (H^+) \quad (9)$$

Substitution of Eqs. (7) and (8) into Eq. (9) leads to the following quadratic equation:

$$Q = \frac{2K_{H_3His^{2+}/H_2His^+}[H_3His^{2+}]}{[H_2His^+]^2} \cdot (H_2His^+)^2 + \left(1 + \frac{K_{H^+/H_2His^+}[H^+]}{[H_2His^+]}\right) \cdot (H_2His^+) \quad (10)$$

Solution of Eq. (10) gives the concentration of H_2His^+ in the stationary phase. The expressions for (H_2His^+) can be substituted into Eq. (4), and the final forms of the model for mono- (Eq. 11) and divalent (Eq. 12) cations are obtained:

$$D_{M^+} = \frac{v_o k'}{v_{st}} = K_{M^+/H_2His^+} \cdot \left(\frac{\sqrt{p^2+q} - p}{4K_{H_3His^{2+}/H_2His^+}[H_3His^{2+}]}\right) \quad (11)$$

$$D_{M^{2+}} = \frac{v_o k'}{v_{st}} = K_{M^{2+}/H_2His^+} \cdot \left(\frac{\sqrt{p^2+q} - p}{4K_{H_3His^{2+}/H_2His^+}[H_3His^{2+}]}\right)^2 \quad (12)$$

where

$$p = [H_2His^+] + K_{H^+/H_2His^+}[H^+]$$

$$q = 8K_{H_3His^{2+}/H_2His^+} Q [H_3His^{2+}]$$

and v_o is the void volume, v_{st} is the volume of the stationary phase. The molar concentrations of the three competing cations in the eluent ($[H^+]$, $[H_2His^+]$, $[H_3His^{2+}]$) can be easily calculated from the c_{His} by the molar fraction (Φ) of species using protonation constant and the actual pH of the mobile phase, e.g., $[H_3His^{2+}] = \Phi_3^{2+} c_{His}$, where $\Phi_3^{2+} = K_1K_2K_3[H^+]^3/(1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3)$.

3. Experimental

3.1. Reagents and solutions

Eluents were prepared by using analytical grade L-histidine monohydrochloride monohydrate (Fluka, Buchs, Switzerland). Ion-exchanged water was purified by a Milli-Q Plus System (Millipore, Bedford, MA, USA) containing a 0.45-μm filter at the outlet. The actual pH was monitored after the eluent was degassed, and the pH was controlled (Orion Model 420A, USA) by addition of diluted HCl (Merck Suprapur). The measurement of pH was carried out at 25°C using an Orion Triode pH electrode, Model 9157 BN (Orion Res., Boston, MA, USA) with internal reference system and built in thermistor. For each eluent and sample composition a number of pH values were controlled, ranging from 1.00±0.02 (with glycine–hydrochloric acid buffer solution) to 4.01±0.02 (potassium hydrogen phthalate) after multiple point calibration. Sample solutions of cations were prepared by dissolution of chloride salts (Baker Deventer, The Netherlands) with the exception of magnesium, which was prepared from sulfate. Stock solutions of the individual alkali- and alkaline-metal cations were prepared with polypropylene volumetric apparatus.

3.2. Instrumentation

A Model 2010i Dionex (Sunnyvale, CA, USA) chromatograph was used in this work. The major components of this system were a high-pressure metal-free pump, 100-μl sample loop, a CG3 cation-exchange guard- (50×4 mm), an IonPac CS3 cation separator column (250×4 mm), (ion-exchange capacity: 100 μequiv./column), CMMS-1 micromembrane eluent suppressor, a CDM conductivity detector and SP 4270 data module integrator. All samples were analyzed in triplicate with a flow-rate of 1.0 ml/min. Pneumatic pressure was used to pump the regenerant solution of tetramethyl-ammonium hydroxide, 70 m*M* at a flow-rate of 5 ml/min.

4. Results and discussion

4.1. Determination of the selectivity coefficients

Modeling of retention behaviour with Eqs. (11)

and (12) requires that ion-specific selectivity coefficients (K_M) and the inter-eluent selectivity coefficients (K_E) be known. These unknowns can be evaluated by measuring retention data at varying eluent concentration and pH. Both the concentration of histidine species and pH of the eluent have been varied in a practical range of 3–6 m*M* His at strong acidic interval within the pH range 1–2. The samples were studied at five eluent concentrations ($c_{\mathrm{His}} = 3.0$, 3.8, 4.5, 5.2 and 6.0 m*M*) at five pH (1.29, 1.38, 1.53, 1.65 and 1.89), i.e., at 25 different eluent compositions. Thus, 25 retention data were obtained systematically for each analyte ions. Fig. 2 shows the eluent compositions used in the experimental procedure. Activities instead of concentrations were used in the actual data processing. The activities of each eluent species were calculated by the extended Debye-Hückel [12] equation, in which the value of the activity coefficient (γ) is expressed as the product of the ionic strength and the charge of the ion. The use of activities is particularly important in the case of H_3His^{2+}, which has a greater impact on the separation ($a_{H_3His^{2+}} = \gamma_3 \Phi_3^{2+} c_{\mathrm{His}}$).

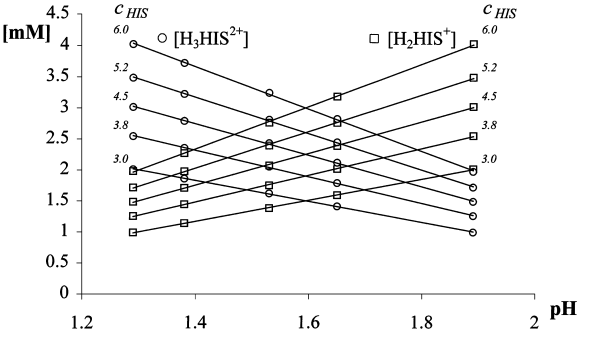

Fig. 2. Eluent compositions used in separations. Mono- and divalent molar fractions ($[H_2His^+]$, $([H_3His^{2+}])$ of histidine (c_{His}) in eluents of known pH. Activities instead of concentrations were used for actual data processing in Eqs. (11) and (12). ($a_{H_2His^+} = \gamma_2 \Phi_2^+ c_{\mathrm{His}}$, $a_{H_3His^{2+}} = \gamma_3 \Phi_3^{2+} c_{\mathrm{His}}$).

Modeling and prediction of retention behaviour require that selectivity coefficients be known. Observed k' values, eluent composition, and pH can be substituted into retention equation for each set studied, thereby producing a sequence of equations in which the selectivity coefficients are the only unknowns. On the basis of retention equation (Eqs. (11) and (12)) derived from ion-exchange equilibria, the selectivity coefficients were by iterative calculation with variability of the coefficients. During the computation, the sum of squares of the difference between the calculated and measured retention data was minimized. A Simplex method (due to Nelder and Mead [13]) was applied to solve this multiple nonlinear regression problem. The resultant values of the calculated selectivity data are shown in Table 1, in which the coefficients were obtained from five retention volumes, measured at five different pH values belonging to the same eluent concentration, and then the values were averaged using the data of various eluent concentrations for all analytes (Table 2). The standard deviations are relatively low, indicating that the coefficients are independent of eluent concentration. Table 2 summarizes the values involve the effect of the 25 data for ion-specific coefficients and all the 100 data evaluated for intereluent coefficients. All coefficients for separated ions (K_M) reflect the observed elution order of Na, K, Mg and Ca.

Table 1
Ion-specific and intereluent selectivity coefficients for alkali and alkaline-earth cations at different eluent concentrations

Eluent concentration (m*M*)	Selectivity coefficients (*K*)											
	Na^+			K^+			Mg^{2+}			Ca^{2+}		
	Na/H_2His	H_3His/H_2His	H/H_2His	K/H_2His	H_3His/H_2His	H/H_2His	Mg/H_2His	H_3His/H_2His	H/H_2His	Ca/H_2His	H_3His/H_2His	H/H_2His
3.0	0.29	10.70	0.45	0.56	10.56	0.45	7.01	10.67	0.45	14.99	11.00	0.45
3.8	0.30	10.74	0.47	0.59	10.98	0.52	7.00	10.63	0.49	14.89	10.76	0.51
4.5	0.28	11.00	0.45	0.56	10.99	0.50	7.52	10.70	0.55	15.32	10.01	0.55
5.2	0.29	10.70	0.55	0.55	10.03	0.55	7.53	10.61	0.55	15.06	10.00	0.55
6.0	0.29	11.00	0.48	0.55	10.81	0.55	7.38	10.00	0.55	15.41	10.01	0.55
Mean±SD	0.29±0.01	10.83±0.16	0.48±0.04	0.56±0.02	10.67±0.40	0.51±0.04	7.29±0.26	10.52±0.29	0.52±0.05	15.13±0.22	10.36±0.49	0.52±0.04

Table 2
Selectivity data obtained from the experimental results by iterative calculations

Analyte	Selectivity coefficients		
	K_{M/H_2His^+}	$K_{H_3His^{2+}/H_2His^+}$	K_{H^+/H_2His^+}
Na^+	0.29±0.01	10.83±0.16	0.48±0.04
K^+	0.56±0.02	10.67±0.40	0.51±0.04
Mg^{2+}	7.29±0.26	10.52±0.29	0.52±0.05
Ca^{2+}	15.13±0.22	10.36±0.49	0.52±0.04
Mean±SD		10.60±0.37	0.51±0.04

The intereluent selectivity constants (K_E) determined at different analyte ions do not deviate from one another confirming the expectation that the constants are independent of ions to be separated. The different values of $K_{H_3His^{2+}/H_2His^+}$ and K_{H^+/H_2His^+} (10.60 vs. 0.51) which reflect the selectivity difference between the divalent and monovalent eluent species, confirm that the H_3His^{2+} species plays an important role in the chromatographic separation.

4.2. Prediction of retention data

Given the selectivity coefficients from Table 2 and the eluent composition, retention times can be calculated. The agreement between the measured and calculated retention time and log k' values is summarized and demonstrated in Table 3 and Fig. 3. Table 3 shows the individual deviations between the $t_{R(meas.)}$ and $t_{R(calc.)}$ for alkali and alkaline-earth ions. These values are given in the last column of table for each analytes at different eluent conditions. If the values of Δ(%) are now averaged for all analyte ions at different eluent compositions (pH, c_{His}), the

Table 3
Comparison of measured and calculated retention times[a]

c_{His} (mM)	pH	$t_{R(meas.)}$ (min)				$t_{R(calc.)}$ (min)				$\lvert\Delta\rvert$ (%)
		Na^+	K^+	Mg^{2+}	Ca^{2+}	Na^+	K^+	Mg^{2+}	Ca^{2+}	
3.0	1.89	2.54	3.10	14.73	28.87	2.52	3.06	14.41	27.97	1.85
3.0	1.65	2.43	2.86	9.94	18.52	2.41	2.85	10.05	18.86	1.03
3.0	1.53	2.34	2.73	8.70	16.00	2.36	2.75	8.46	15.55	1.78
3.0	1.38	2.31	2.69	7.46	13.43	2.31	2.65	6.80	12.10	5.00
3.0	1.29	2.35	2.73	6.97	12.26	2.28	2.58	5.93	10.27	9.90
3.8	1.89	2.49	3.02	12.34	23.62	2.47	2.96	12.24	23.30	1.24
3.8	1.65	2.40	2.77	8.39	15.37	2.37	2.77	8.79	16.15	2.77
3.8	1.53	2.33	2.69	7.22	12.86	2.33	2.69	7.53	13.54	2.40
3.8	1.38	2.31	2.66	6.39	11.08	2.29	2.60	6.21	10.80	2.11
3.8	1.29	–	–	5.95	10.19	2.26	1.52	5.49	9.31	8.15
4.5	1.89	2.37	2.85	11.32	22.63	2.43	2.89	10.91	20.66	4.06
4.5	1.65	2.32	2.65	7.80	14.24	2.34	2.72	8.00	14.60	2.09
4.5	1.53	2.32	2.64	6.65	11.76	2.31	2.65	6.93	12.36	2.53
4.5	1.38	2.28	2.58	5.88	10.09	2.27	2.57	5.81	10.03	0.68
4.5	1.29	2.26	2.56	5.52	9.30	2.24	2.52	5.18	8.72	3.80
5.2	1.89	2.41	2.87	10.75	20.61	2.40	2.83	9.91	18.47	5.00
5.2	1.65	2.34	2.66	7.14	12.79	2.32	2.68	7.39	13.23	2.12
5.2	1.53	2.29	2.57	6.15	10.68	2.29	2.61	6.46	11.32	2.58
5.2	1.38	2.21	2.50	5.16	8.65	2.25	2.54	5.49	9.30	4.32
5.2	1.29	–	–	4.97	8.05	2.22	2.49	4.95	8.18	1.00
6.0	1.89	2.37	2.79	10.04	19.33	2.37	2.78	9.02	16.72	5.90
6.0	1.65	2.28	2.59	6.50	11.53	2.30	2.64	6.84	12.16	3.36
6.0	1.53	2.24	2.51	5.62	9.63	2.27	2.58	6.03	10.49	4.90
6.0	1.38	2.25	2.50	4.96	8.18	2.24	2.51	5.19	8.72	3.02
6.0	1.29	–	–	4.65	7.53	2.22	2.47	4.72	7.74	2.15
										Mean 3.35%

[a] Flow-rate of 1 ml/min, $|\Delta| = 100\ |t_{R(calc.)} - t_{R(meas.)}|/t_{R(meas.)}$.

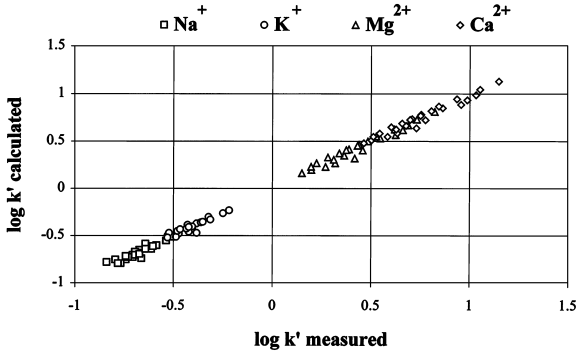

Fig. 3. Relationship of measured and calculated capacity factors for alkali and alkaline earth ions eluted with acidified histidine (slope, 0.993; intercept, −0.003; correlation coefficient, 0.997 for 100 data pairs).

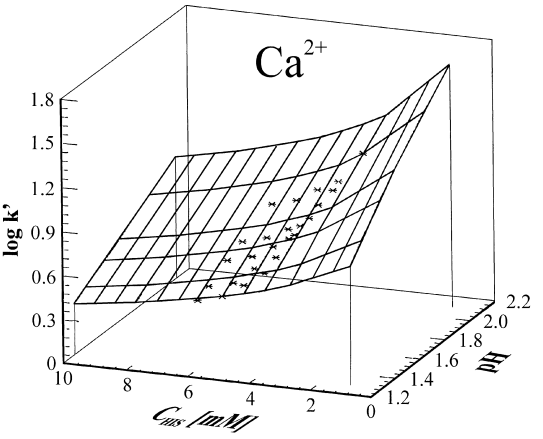

Fig. 5. Calculated retention surface and measured retention data points (*) for Ca^{2+} ions.

retention model gives sufficiently reliable data of retention times to serve as the basis of a possible optimization procedure for eluent composition. The averaged mean of errors is 3.35%, which is the magnitude of the good experimental precision. This conclusion is also supported by the good agreement between the measured and calculated capacity factors for all cations in Fig. 3. The slope of the $\log k'_{\text{calc.}}$ versus $\log k'_{\text{meas.}}$ function is 0.993. The correlation coefficient calculated for 100 data pairs is 0.997.

If we use a relatively large number of data points we can predict retention behavior in the wide range of eluent pH and concentration. Figs. 4–7 demonstrate the calculated retention surfaces for cations studied in this work and show the effects of eluent conditions simultaneously. The results of retention modeling in three-dimensional surfaces helped us to provide a detailed understanding of the chromatographic processes. The effect of pH on the retention is based on the change in the molar ratio of the eluent components and Fig. 4 indicates that the retention is influenced most strongly by pH. Since acidity in the separation was varied in the range pH 1.0–2.0, the increase in the molar fraction of H_3His^{2+} in the eluent is more than 3 times on decreasing the pH. The change in the molar ratio of the eluent components as a function of pH results in a significant change in the capacity factor for all analyte cations. This effect can be attributed to the stronger eluent power of divalent component of the eluent, i.e., higher selectivity coefficients of H_3His^{2+} than that of H_2His^{+} (see Table 2). Separation of cations using histidine in partially protonated state is

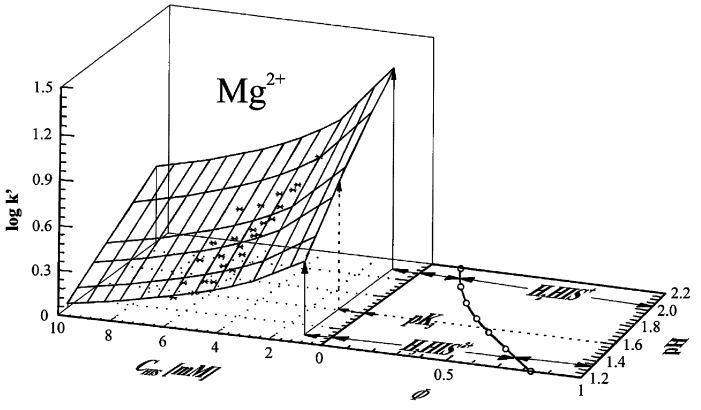

Fig. 4. Calculated retention surface and measured retention data points (*) for Mg^{2+} ions eluted with histidine. Partial molar fractions of eluent components are also illustrated as functions of pH.

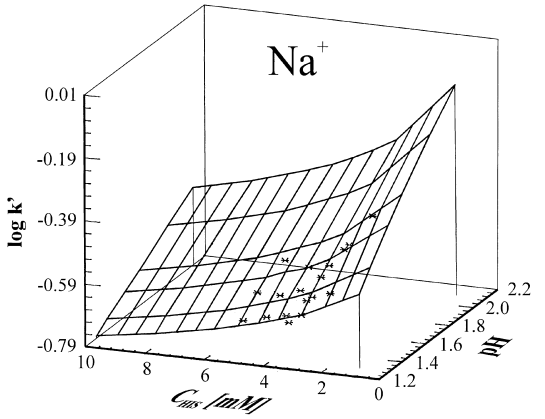

Fig. 6. Calculated retention surface and measured retention data points (*) for Na^+ ions.

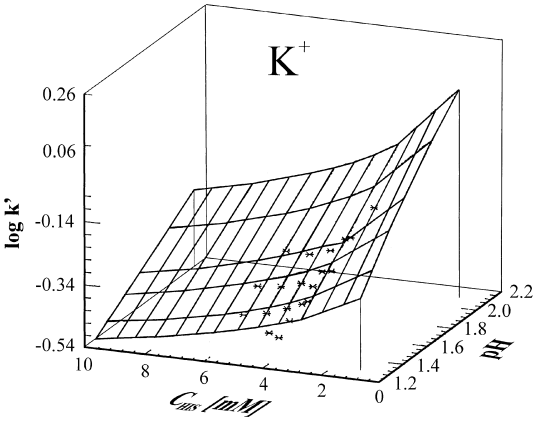

Fig. 7. Calculated retention surface and measured retention data points (*) for K^+ ions.

a powerful approach. The price to be paid for this capability to manipulate retention is the greater sensitivity of k' to small changes in pH, when eluent pH is near pK, especially at low level in eluent concentration, as seen in Fig. 4. Consequently, the precise and accurate pH control is essential. A further consequence is that the capacity factor for the divalent analytes (Mg^{2+}, Ca^{2+}) decrease faster (because of the power of 2) than those for monovalent cations (Na^+, K^+) with changing pH (see Eqs. (11) and (12)).

5. Conclusion

The versatility of cation chromatography has been developed by the application of multispecies dipolar eluent. This method simplified the mono- and divalent cation analysis by suppressor-based IC. The elution behaviors of alkali and alkaline earth cations are dependent on the eluent concentration and pH of acidified L-histidine, systematically. The major factor in the control of selectivity for this system is the imposition of protonation equilibria of dipolar histidine eluent on the ion-exchange distribution process. The composition of the eluent and the selectivity of the system can be easily governed by the eluent pH. From the chemical equilibria and selectivity data (K_{M/H_2His^+}, $K_{H_3His^{2+}/H_2His^+}$, K_{H^+/H_2His^+}) it is evident that the separations occur effectively at lower than pH 2 and detection pH close to the pI value of histidine. The multispecies eluent retention model has been derived and applied to cation separation which has provided experimental verification of its ability to correlate and predict retention data in cation chromatography.

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