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Short communication

Histidine as a dipolar eluent component in cation chromatography

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Abstract

Acidified L-histidine [2-amino-3-(4-imidazolyl)propionic acid] was found as an eluent in membrane-suppressed cation chromatography. Both mono- and divalent cations such as alkali and alkaline-earth metals can be separated by simple isocratic chromatography using conductivity detection. The effect of eluent pH and concentration on the retention was determined. The elution and suppression behavior can be easily governed by the pH of the mobile phase. The eluent exists as the divalent driving cation at lower a pH than 2, with the ability to elute strongly retained ions, and has very low conductivity background at the pH of the isoelectric point of histidine. Results are compared with another combination of 2,3-diamino-propionic acid eluent as a dipolar component. The applicability of the phase system is demonstrated by the analysis of a high purity water sample. © 1997 Elsevier Science B.V.

Keywords: Mobile phase composition; Histidine; Metal cation; Alkali metals; Alkaline earth metals

1. Introduction

The separation of cations in the chromatographic system are affected by relative sample and eluent charge, eluent concentration and the pH of the mobile phase [1]. The eluent is a powerful parameter that can be varied when devising cation separations. The most commonly used eluents in cation chromatography are the mineral acids. Solutions in the range of 1–10 m*M* readily elute alkali metals, ammonium and organic amines. The hydrogen (hydronium) ion is an excellent choice for use as eluent because it is converted to water in the suppressor device before conductivity detection. However, it has a low ability to displace polyvalent cations, such as the alkaline-earth ions. Therefore, the elution of both monovalent and divalent cations cannot be obtained without either modifying the eluent or the column during a single run. Both alkali and alkaline-earth metals can only be eluted in a single injection using either gradient elution or column switching [2]. For a number of years, commonly used eluents were the protonated cations of aromatic and heterocyclic amines [3] in nonsuppressed ion chromatography. Because some of these are toxic and carcinogenic, they are no longer used. Ethylenediamine salts are also effective eluents for divalent cations because of their dual charge in the diprotonated form (EnH_2^{2+}) in the acidic pH range [4,5]. In suppressed cation chromatography silver and cupric ions were also employed as eluent components; silver nitrate eluent is suppressed by a combination of ion exchange and precipitation, while the copper eluents are suppressed by complexation [6]. Similarly, zinc eluents can be removed by precipitation using a suppressor in the hydroxide form [4]. Basic amino acids appear to be the most promising eluent components for cation separation, while the inorganic eluent ions have only moderate sensitivity and poor selectivity. Recently, acidified 2,3-diaminopropionic acid (DAP) [2] has become widely used. The membrane suppressor converts the amino acid substance to its zwitterionic form, which has no conductivity.

This paper presents a comprehensive evaluation of

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the elution and suppression properties of histidine as a possible dipolar eluent component. Factors affecting the use of acidified histidine in a membrane-suppressed cation chromatographic system are also shown in this paper.

2. Theory

The L-histidine [2-amino-3-(4-imidazolyl)propionic acid] molecule protonated with HCl can form mono- and divalent cations. For the formation of histidine cations, three equilibrium constants must be considered. These are $K_1=10^{9.10}$, $K_2=10^{6.02}$ and $K_3=10^{1.60}$ [7].

The proton ionisation equilibria may be represented:

$$HIS^- \underset{+H^+}{\overset{K_1}{\Leftrightarrow}} HHIS \underset{+H^+}{\overset{K_2}{\Leftrightarrow}} H_2HIS^+ \underset{+H^+}{\overset{K_3}{\Leftrightarrow}} H_3HIS^{2+} \quad (1)$$

The basic amino acid, such as histidine, contains two amino groups and one carboxylic group is doubly positive at low pH interval. From the ionisation equilibria (see Fig. 1) it is evident that this occurs at lower than pH 1.6 and the monovalent species exist between pH 1.6 and 6. Knowing the disposition of charge of the histidine as a function of pH of the mobile phase is important to an understanding of their elution and suppression capability. At the eluent pH values used —practically between 1 and 3— three eluent species will be present: hydrogen ions, monovalent H_2HIS^+ and divalent H_3HIS^{2+} ions. Thus, the composition of such an eluent can be easily governed by its pH. The ion-exchange processes for M^+ analyte ions in the elution and suppression are given by Eq. (2),

$$\begin{array}{ccc} R-SO_3H_2HIS + M^+ & \overset{K_{M/H_2HIS}}{\Leftrightarrow} & R-SO_3M + H_2HIS^+ \\ \Updownarrow H^+ & & \Updownarrow OH^- \text{ suppression} \\ H_3HIS^{2+} & & HHIS + H_2O \\ \text{elution} & & \text{detection} \\ (1<\text{pH}<3) & & (\text{pH}\cong 7.56 = \text{p}I) \end{array} \quad (2)$$

where R-SO_3^- denotes the cation-exchange stationary phase and K_{M/H_2HIS} is the ion-exchange equilibrium constant. Histidine is much more powerful than an eluent containing only hydrogen ions. Eluents of this type have the dual advantage of high elution power because of their higher charge and ability to elute more strongly retained ions with an eluent component which is convertible to the isoelectric form. The isoelectric point (pI) of HHIS species is reached

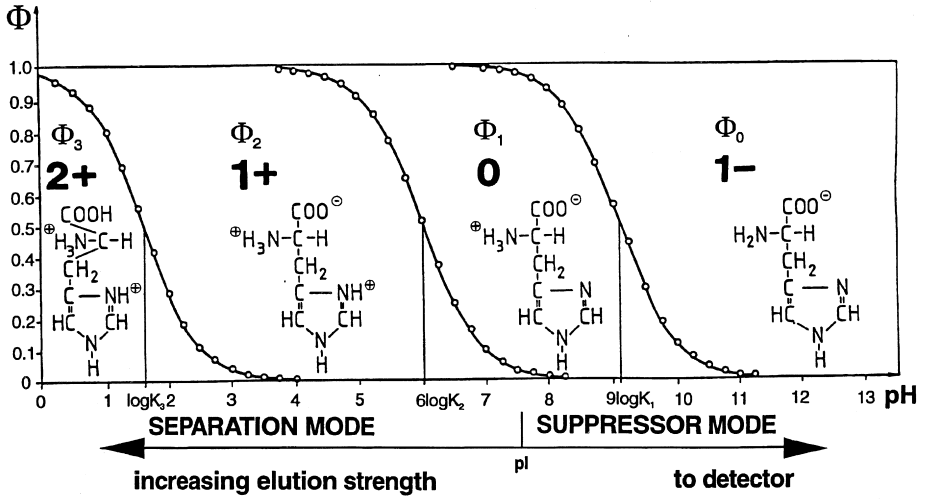

Fig. 1. The proton ionization equilibria of L-histidine eluent. Calculated variation with pH of the composition of a HCl–histidine system. Φ is the partial molar fractions of histidine species ($\Phi_0+\Phi_1+\Phi_2+\Phi_3=1$).

when the total equivalents of protonated amino forms balance the one equivalent of the carboxylate group. Since the p*I* represents a point of neutrality, it also represents a point of minimum ion-exchange activity and conductivity. In order to achieve efficient suppression by the anion-exchange membrane in the hydroxide form, this p*I* should be as low as possible. The isoelectric pH can be determined by the mean value of the amine and carboxylic ionization constants. The isoelectric point of histidine (p$I=7.56$) is much lower than those of the other basic amino acids. Other eluents which can be used in suppressed cation chromatography are arginine (p$I=10.76$), lysine (p$I=9.47$) and diaminopropionic acid (p$I=8.15$).

3. Experimental

3.1. Reagents and solutions

Eluents were prepared by using analytical grade L-histidine monohydrochloride monohydrate and L-2,3-diaminopropionic acid monohydrochloride (Fluka, Buchs, Switzerland). Distilled water was purified using 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). Sample solutions of cations were prepared by dissolution of chloride salts (Baker Analyzed Reagents, The Netherlands) with the exception of magnesium, which was prepared from sulfate. Stock solutions of the individual alkali and alkaline-earth 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, a 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 an 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 tetramethylammonium hydroxide at a flow-rate of 5 ml/min.

4. Results and discussion

4.1. Typical separation

Fig. 2 shows the chromatograms obtained for alkali and alkaline-earth metal ions by using acidified histidine eluents. Both mono- and divalent cations can be eluted in a single isocratic run. For this reason, retention can be considered to be optimized if the eluent concentration falls within the range $3 \leq [HIS] \leq 8$ m*M* at pH below 2. As shown in Fig. 2, relatively low retention times and adequate resolution of six cations was obtained. Two points of interest emerge from Fig. 2. First, the elution order of cations (Fig. 2a) is identical in comparison to that obtained with the DAP eluent by gradient technique [2]. Secondly, the eluent also provided a superior conductivity signal for alkaline-earth ions (Fig. 2b), which is a consequence of the suppressibility of histidine. The membrane suppressor includes an anion-exchange material in hydroxide form to minimize background conductivity and to enhance analyte response. The cation analytes are converted in the suppressor to alkali and alkaline hydroxide, the acid is neutralized by hydroxide and the histidine cations are converted into isoelectric form, which has no conductivity. The only requirement is that the pH be close to 7.5 in the effluent, and this must always be maintained by control of the regenerant flow-rate in the suppressor. The pH of 7.5 ensures neutralization of acid, the isoelectric form of histidine and their minimum conductivity. Suppressor regenerant was 50 m*M* tetramethylammonium hydroxide.

4.2. Factors affecting the use of histidine eluent

In order to effectively characterize cation separations, we must also consider the factors that determine histidine eluent driving strength. The

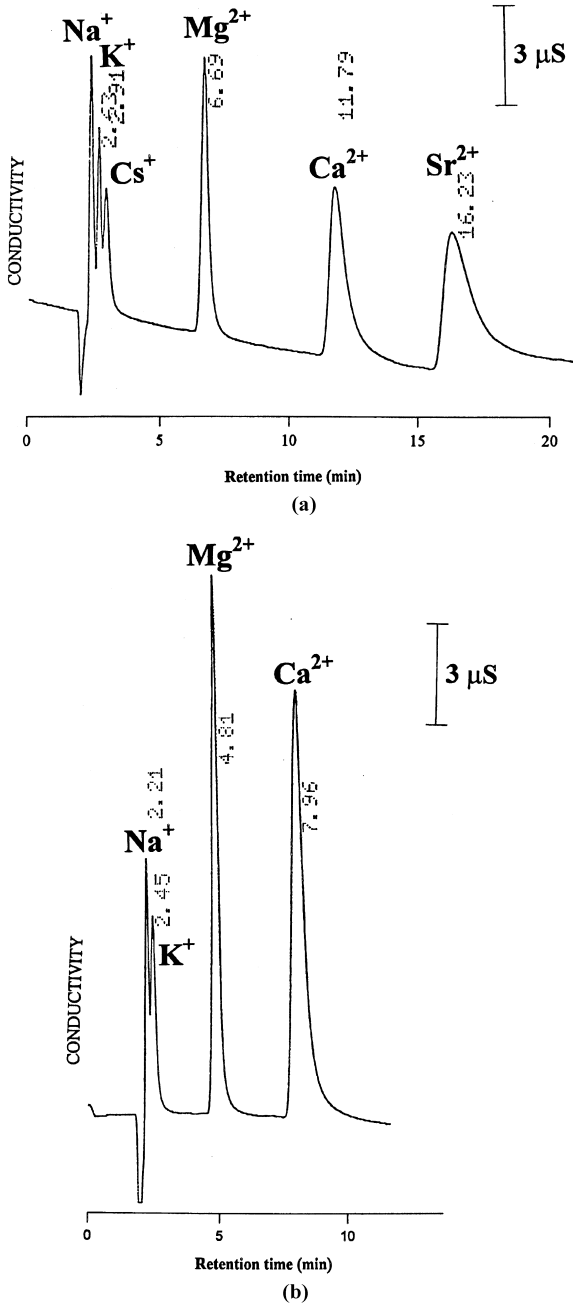

Fig. 2. Isocratic separation of mono- and divalent inorganic cations with eluent: (a) 4.5 m*M* histidine, 27.5 m*M* HCl; and (b) 8.0 m*M* histidine, 27.5 m*M* HCl using suppressed conductivity detection. Sample peaks: $Na^+=2$, $K^+=2$, $Cs^+=3$, $Mg^{2+}=4$, $Ca^{2+}=4$, $Sr^{2+}=12$ mg/l.

capacity factor of alkali and alkaline-earth ions was determined as a function of the histidine concentration and the pH of the mobile phase (Fig. 3). All of the results shown were obtained from a sample loop of 100 μl of a mixture containing 2–4 mg/l of each of the cations. Fig. 3 shows that pH and eluent concentration are powerful parameters for adjusting the retention. The effect of driving strength on sample ion retention depends on the charge of the cations. Thus, the retention time of a divalent sample ion changes more rapidly in response to changes in eluent composition than does the retention time of a monovalent ion. However, the order of elution for mono- and divalent cations is independent of the histidine composition. The capacity factors for cations show an increase as the eluent pH is raised and the cationic charge on the histidine is decreased. The effect of pH on the retention is evident from the protolytic equilibria of the histidine eluent (see Fig. 1).

The results of variation of cationic charge with eluent pH for the histidine eluent is illustrated in Fig. 4a–c, which show comparative chromatograms obtained by using 4.5 m*M* histidine at three different pH values as the representative eluents. It can be seen that eluents of any desired effective cationic charge between (+1) and (+2) can be obtained, so that a wide choice in eluent strengths is possible. The eluent used in Fig. 4a is 27.5 m*M* HCl, 4.5 m*M* histidine. At this pH (1.53) the ratio of divalent to monovalent HIS is 58% H_3HIS^{2+} and 42% H_2HIS^+. The alkaline-earth ions could not be eluted under the chromatographic conditions used in Fig. 4c. At this pH (2.32) the ratio is much greater for the monovalent (84%) than for the divalent driving cation (16%). Practically, at $pH>\log K_3$ divalent cations are too strongly retained to permit the determination of the retention data. These results indicated that the counter ion concentration in combination with the pH is a vital parameter in determining the characteristics of the eluent. Chromatograms obtained with the other combination of dipolar eluents studied, namely DAP, were similar, and comparative examples are shown in Fig. 5. It is interesting to note that an eluent containing DAP+HIS in identical molarities (2.25 m*M*) seems to be the best with respect to peak symmetry, efficiency and speed of

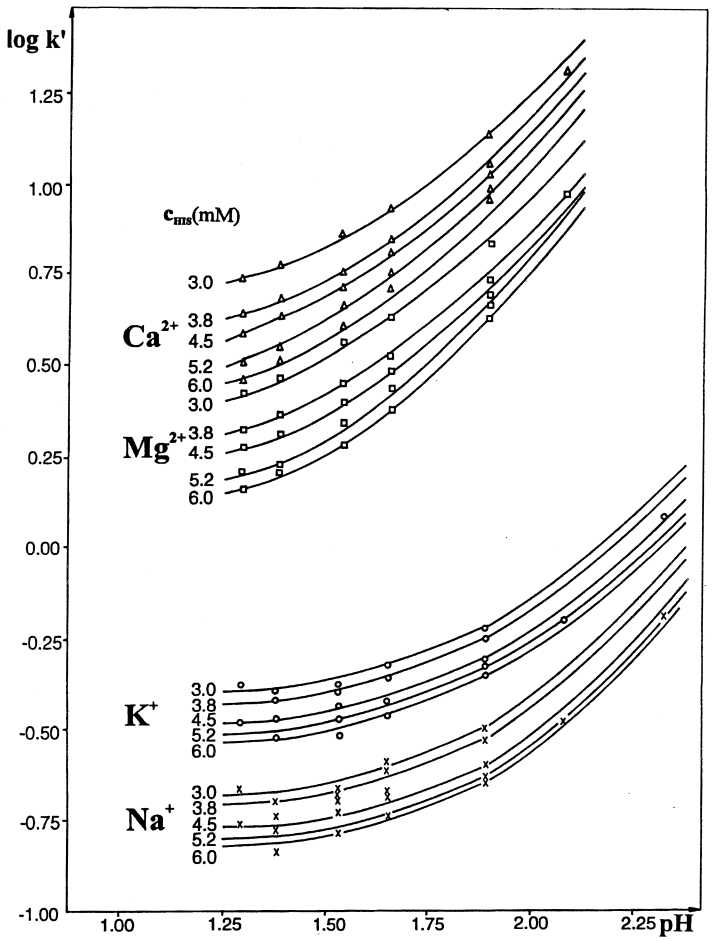

Fig. 3. Effect of eluent pH and concentration on the retention behavior of inorganic cations.

separation. Table 1. lists the retention data obtained for representative eluents of each type.

4.3. Application

The applicability of the described phase system is demonstrated in Fig. 6, which shows the trace analysis of cations in secondary coolant water from nuclear power reactor. The eluent (27.5 m*M* HCl + 2.25 m*M* HIS + 2.25 m*M* DAP) has been successfully employed in quantification of alkali and alkaline-earth ions at μg/l concentration levels. For all cases the linear analytical range, from 20 to 2000 μg/l, is conveniently observed without a preconcentration step.

5. Conclusion

This study has shown that acidified histidine can be applied in the simultaneous separation of alkali and alkaline-earth metals using membrane-suppressed conductivity detection. The main advantages of the described phase system are its simplicity and detectability since the separations can be performed under isocratic conditions, and the suppressor con-

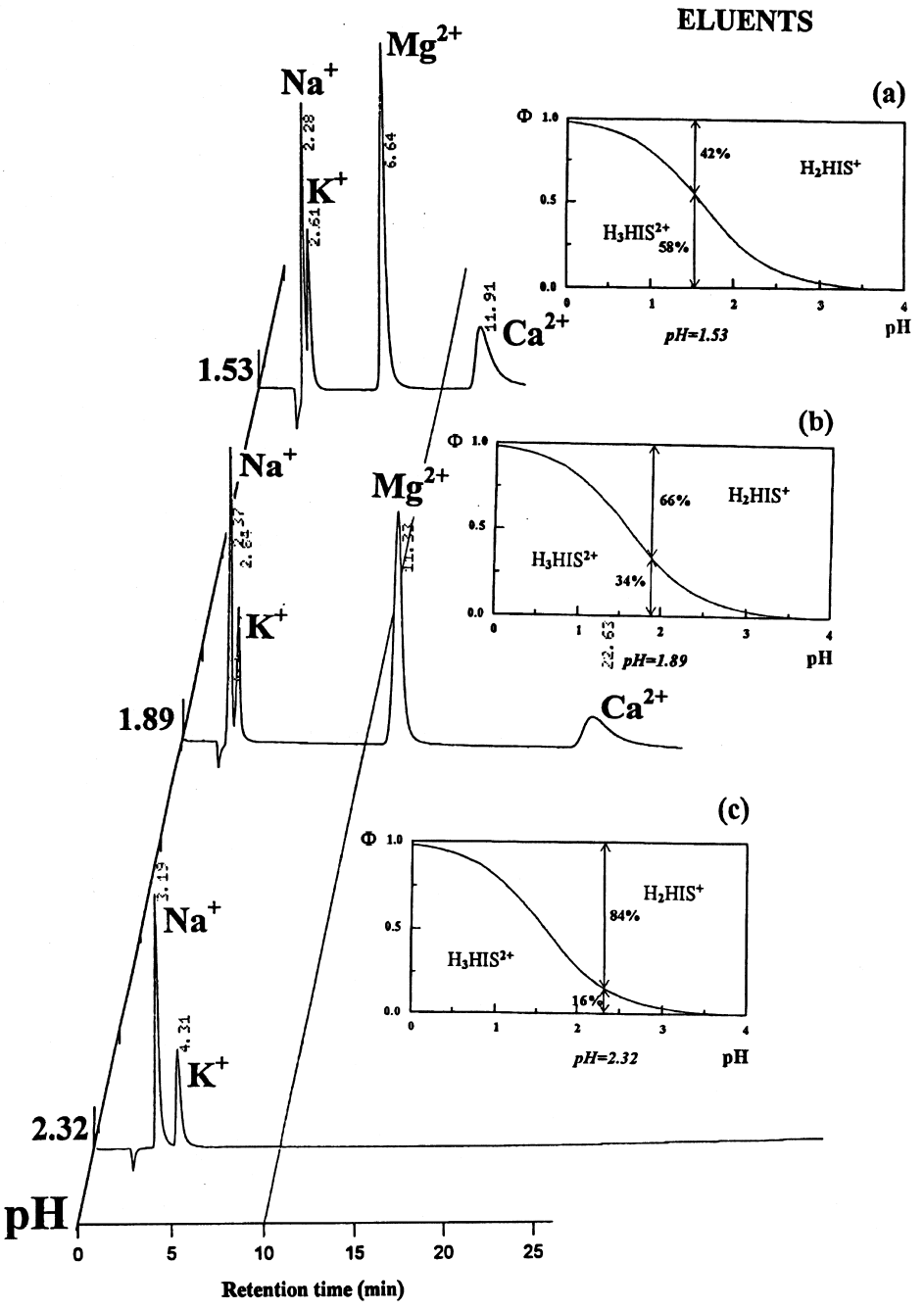

Fig. 4. Comparative chromatograms of cations using a variety of pH values at 4.5 m*M* histidine concentration with eluent: (a) 27.5 m*M* HCl, 4.5 m*M* HIS, pH 1.53; (b) 10.0 m*M* HCl, 4.5 m*M* HIS, pH 1.89; (c) 1.0 m*M* HCl, 4.5 m*M* HIS, pH 2.32.

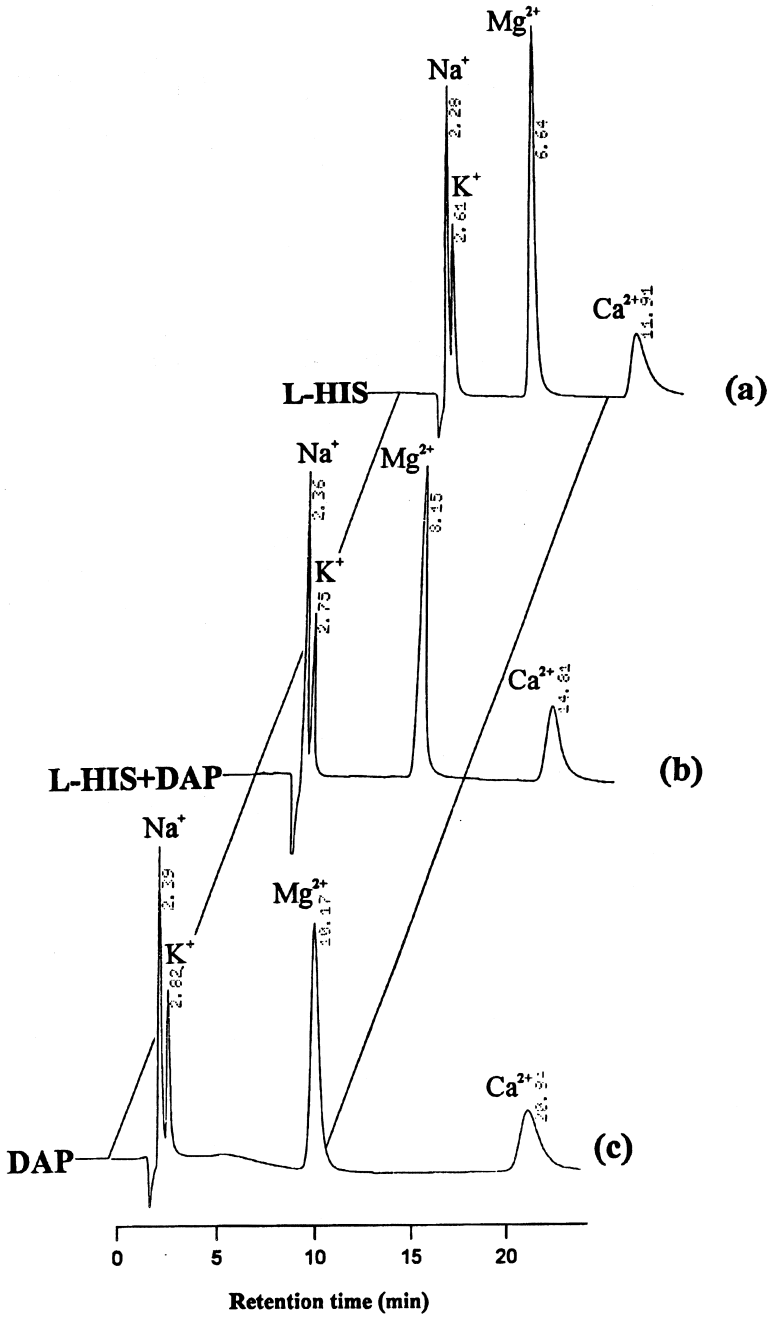

Fig. 5. Comparative chromatograms of cations using a variety of the type of dipolar components with eluent: (a) 27.5 m*M* HCl, 4.5 m*M* HIS; (b) 27.5 m*M* HCl, 2.25 m*M* HIS, 2.25 m*M* DAP; (c) 27.5 m*M* HCl, 4.5 m*M* DAP.

verts them to their dipolar forms, which have no conductivity. The separation of cations can easily be governed by changing the eluent composition. Future work will be devoted to the application of a theoretical model to describe and predict retention behavior as the function of eluent parameters in this system.

Table 1
Retention times of alkali and alkaline-earth metals obtained with typical dipolar eluents

Eluent	Composition (m*M*)	pH	Retention time (min)			
			Na^+	K^+	Mg^{2+}	Ca^{2+}
HCl	32.0	1.49	3.70	5.47	—[a]	—
HCl + HIS	1.0 + 4.5	2.32	3.19	4.31	—[a]	—
HCl + HIS	10.0 + 4.5	1.89	2.37	2.85	11.32	22.63
HCl + HIS	27.5 + 4.5	1.53	2.29	2.62	6.65	11.94
HCl + DAP	27.5 + 4.5	1.55	2.39	2.82	10.17	20.84
HCl + HIS + DAP	27.5 + 2.25 + 2.25	1.54	2.36	2.75	8.15	14.81

[a]No peak.

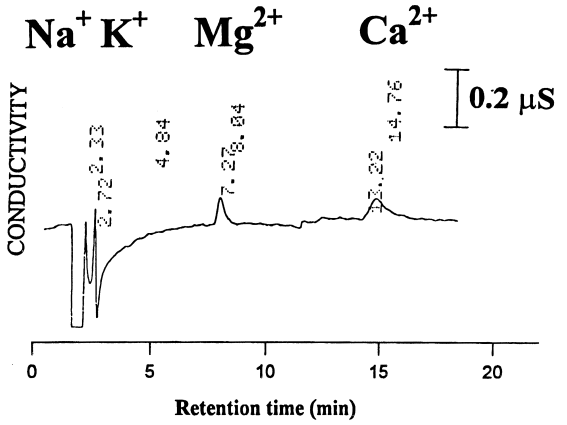

Fig. 6. Cation analysis of high purity water sample. Eluent conditions are as Fig. 5b. Sample peaks: $Na^+ = 30$, $K^+ = 40$, $Mg^{2+} = 30$, $Ca^{2+} = 80$ μg/l. The peak-height method of calibration was used.

Acknowledgements

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