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Capacity gradient anion chromatography with a borate complex as eluent

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Abstract

Complex formation between borate compounds and vicinal diols is well recognized. Generally, in a chemically bonded anion-exchange resin, many hydroxyl groups are introduced on the surface of the resin in order to make the resin hydrophilic. The borate as an eluting reagent also reacts to these hydroxyl groups, and this complex formation decreases the apparent ion-exchange capacity of the column by being dissociated to the anion depending on the eluent pH. In the present work a method is described for the simultaneous determination of anions based on the capacity gradient for suppressed ion chromatography. A Tosoh IC-Anion-PW column and dihydroxyphenylborane–mannitol eluent system were used. To maintain baseline stability, it was helpful to keep the borate concentration constant during a gradient of 16 to 0 m*M* mannitol as a modifier to prevent the complex formation with the hydroxyl on the resin. The chemical composition of the eluents and gradient profiles are discussed and the application to the analysis of the condensed phosphates with widely varying retention times as food additives in a cheese sample is presented.

Keywords: Mobile phase composition; Complexation; Capacity gradients; Food analysis; Phosphates

1. Introduction

Ion chromatography (IC) has been widely recognized as a useful tool for the simultaneous quantitative determination of anions, and it is becoming established as an official method in various fields. IC is classified into two types according to its instrumental design: the one type is suppressed technique equipped with an ingenious apparatus to enhance the sensitivity [1], and the other type is nonsuppressed technique without such an apparatus [2,3]. They are complementary techniques, and thus determining which of the two technique is best for a particular analyte will enhance their usefulness.

One of the most difficult applications of IC is the simultaneous determination of analytes with widely varying retention times. In the non-suppressed technique, gradient elution is not recommended because the change in ionic strength of the eluent directly influences the baseline conductance. Although neutralization with an acidic suppressant must contribute to the baseline stability in the suppressed technique, gradient elution makes the neutralization insufficient. The reason for the baseline instability is that the eluents employed in this technique are confined to weak acids, such as carbonate, that do not have a strong eluting strength and so it is necessary to use them at a high concentration for the elution of strongly bound species. It is unfortunate that the only

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practical eluting species for gradient suppressor IC at present is the hydroxide ion [4–6], whose greatest affinity is for the hydrogen ion. In other words, the hydroxide ion has the weakest eluting strength.

Recently, Lamb and co-workers have explored the capacity gradients in which the gradient separations are achieved by decreasing the ion-exchange capacity of the column during the course of the separation [7–10]. An advantage of this method is the high stability of the baseline owing to very little or no change in the eluent ionic strength. But the gradient analysis by this procedure may not be repeated by everyone. The columns used were prepared by adsorbing macrocyclic ligands onto polymeric resins. However, this procedure may not be available to all laboratories because the macrocyclic ligands are commercially unavailable and difficult to prepare.

Complex formation between borate compounds and vicinal diols is well recognized. In some commercially available, chemically bonded anion-exchange columns, there are many hydroxyl residues on the polymeric resins that make the resins hydrophilic. If borate compounds form anionic complexes with these hydroxyl, the apparent ion-exchange capacity of the column will decrease. In this study we investigate a novel capacity gradient in suppressed IC. One objective was to establish an eluent constitution that controls the borate complex formation with the hydroxyl residues. It is also of interest to apply this gradient elution to the analysis of strongly bound species in actual samples.

2. Experimental

Extra-pure grade dihydroxyphenylborane was purchased from Wako (Osaka, Japan). Pentasodium tripolyphosphate hexahydrate, trisodium trimetaphosphate and hexaammonium tetrapolyphosphate were purchased from Sigma–Aldrich Japan. (Tokyo, Japan). Sodium tetrapolyphosphate containing condensed phosphates with various chain lengths from Nacalai Tesque (Kyoto, Japan) was used as a condensed phosphates mixture. All other reagents used in this work were of guaranteed grade and were purchased from Wako.

The IC system consisted of a Hitachi (Tokyo, Japan) L-6300 low-pressure ternary gradient pump, a Rheodyne (Cotati, CA, USA) 7125 injector, a Shodex (Tokyo, Japan) AO-30C column oven, a Yokogawa Analytical Systems (Musashino, Japan) SA1 suppressor and a Waters (Milford, MA, USA) 432 conductivity detector. Columns used here were a Tosoh (Tokyo, Japan) IC-Anion-PW column (5 cm× 4.6 mm I.D.) packed with polyacrylate-based anion-exchange material with a particle size of 10 μm, and a Dionex (Sunnyvale, CA, USA) AS4A-SC column (25 cm×4 mm I.D.) packed with polyethylvinylbenzene–divinylbenzene substrate with a particle size of 12 μm and anion-exchange latex particles absorbed onto it. Their ion-exchange capacities were specified as 30 μequiv. ml^{-1} and 20 μequiv./column, respectively.

Non-ionic adsorption behaviors of borate on the column resins were investigated by the breakthrough method. The elution profiles of adsorbed solutes from the columns were monitored by a Shodex RI-71 refractive index detector.

Ternary gradient elution with a flow-rate of 1 $ml\,min^{-1}$ at 40°C was used with the following eluents: eluent A, 48 m*M* dihydroxyphenylborane–32 m*M* NaOH; eluent B, 32 m*M* mannitol; eluent C, 0.019 m*M* disodium ethylenediamine tetraacetate (EDTA)–16 m*M* NaOH. The gradient was as follows: isocratic (0–2 min) with 50% A+50% B; linear (2–22 min) to 50% A+0% B+50% C and then these values were held. The suppressant used was 15 m*M* sulfuric acid.

Preparation of the sample solution followed the procedure described previously [11,12], i.e., 5 g of commercially available cheese was homogenized with 4% trichloroacetic acid (TCA); made up to 25 ml with TCA; diluted 25 times with water and ultrafiltrated through a Tosoh Ultracent-10.

3. Results and discussion

In order to investigate the influence of the hydroxyl residue bound to the resin on the elution behavior of an analyte with a borate eluent, the eluting strength of borate on an IC-Anion-PW column, which has many residual hydroxyl groups on the resin surface, was compared with that of a borate–mannitol complex. The existence of surplus mannitol in the eluent was thought to eliminate the

interaction between borate and the hydroxyl residues. An AS4A-SC column was used as a reference, because it presumably has no hydroxyl residues. Chromatograms of chloride on both columns are shown in Fig. 1. The ion concentration and pH of both eluents were adjusted to be almost the same. On the SC column, the borate–mannitol complex showed stronger eluting strength than borate. The main reason for this may be that a fairly large ion radius of hydrated borate was reduced by complexing with mannitol, and it is unlike that the divalent complex of borate–mannitol in a 2:1 molar ratio was formed in the eluent. On the contrary, chloride eluted early from the PW column with the borate eluent, although its ion-exchange capacity is larger than that of the SC column, and the eluting strength of borate is inferior to that of the borate–mannitol complex. The discrepancy in elution behaviors between the two columns may indicate some interaction between borate and the hydroxyl residues on the resin surface of the PW column.

The amount of adsorbed boric acid on the resin surface was measured by the breakthrough method. To prevent its ionic adsorption, both columns were equilibrated with phosphate buffer of 20 m*M* and pH 4.5. Fig. 2 depicts the effluent profiles of boric acid and glycerin as a reference solute after changing the eluent to the phosphate buffers, to which 1 m*M* each of them were added, respectively. There was no difference between the two effluent curves from the SC column. Thus boric acid was not thought to interact with the resin surface as glycerin does. On the other hand, the equilibration with boric acid on the PW column was distinctly retarded in comparison with glycerin, and it took a longer time to reach a plateau. These experimental data support the hypothesis that boric acid complexes with hydroxyl residues on the resin surface. The dissociation of this complex should be stimulated by increasing the pH of the buffer, and the dissociation causes a decrease in the apparent ion-exchange capacity of the PW column.

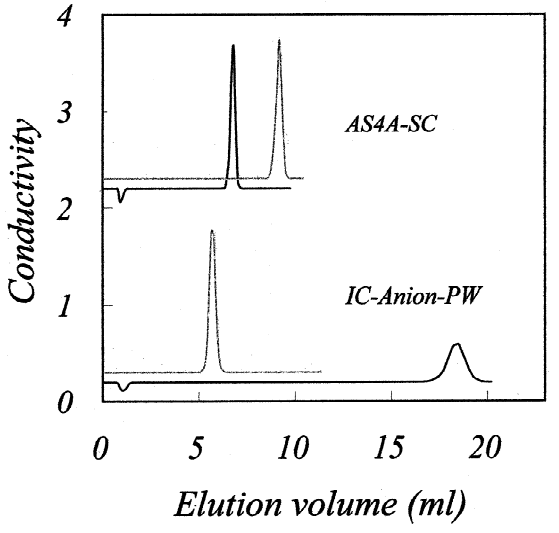

Fig. 1. Effect of D-mannitol in the borate eluent on the elution of chloride for AS4A-SC and IC-Anion-PW columns. Conditions: sample size; 15 μl injection of 0.1 m*M* chloride, eluents; 15 m*M* H_3BO_3–9.2 m*M* NaOH (pH 9.2) for broken lines and 9.2 m*M* H_3BO_3–0.05 *M* D-mannitol–9.2 m*M* NaOH (pH 9.3) for solid lines, suppressant; 15 m*M* H_2SO_4, column temperature; 23–25°C.

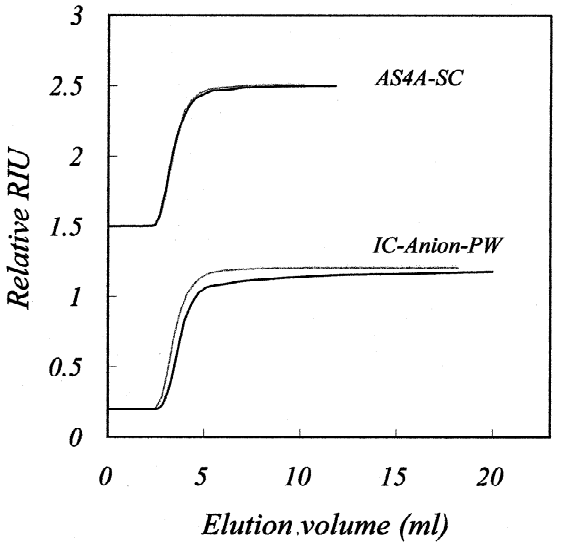

Fig. 2. Elution profiles of boric acid (solid lines) and glycerin (broken lines) from AS4A-SC and IC-Anion-PW columns by the breakthrough method.

Fig. 3 depicts the relationship between the eluent ion concentration and the adjusted retention time of chloride at a constant eluent pH on both log scales. The closed circles represent the retention times in the borate eluent and the open ones represent those in the borate–mannitol system, where mannitol is present in excess. The adjusted retention time is calculated by subtracting time due to the void volume of the system from the actual retention time. The void

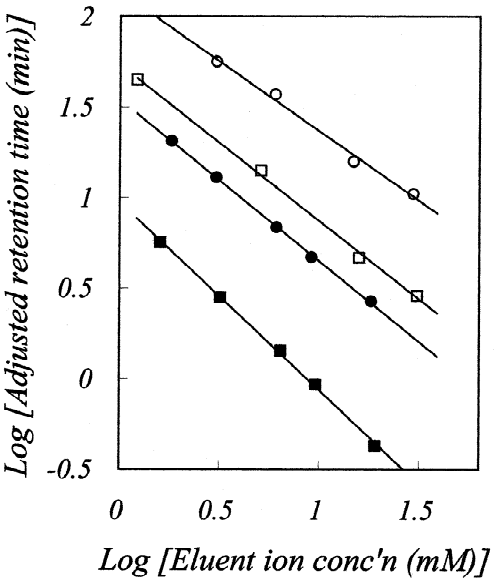

Fig. 3. Plots of log (eluent ion concentration) versus log (adjusted retention time of chloride) around pH 9 on IC-Anion-PW column. Symbols: ■; dihydroxyphenylborate eluent, ●; borate eluent, □; dihydroxyphenylborate–mannitol eluent, ○; borate–mannitol eluent.

volume corresponds to the retention volume of the injection peak. In IC, it is said that the slope of the linear relation between the eluent ionic concentration and the adjusted retention time exhibits the ratio of the charges between the analyte ion and the eluent ion, and the ordinate intercept is in proportion to the capacity of the column [13]. When considering the former result that the eluting strength of borate is weaker than that of the borate–mannitol complex, it seems reasonable to suppose that the ion-exchange capacity of the PW column is an order of magnitude smaller due to formation of the borate complex on its resin surface.

We would like to consider the application of this capacity gradient method to the actual ionic species with widely varying retentions. The eluting strength of borate is, however, too weak to analyze the common ionic species, so its use as an eluent for suppressed IC is confined to the elution of weakly bound analytes [14]. An ionic species with high hydrophobicity shows strong eluting strength in IC by following the basic principle for nonsuppressed IC [15]. The eluting strength of dihydroxyphenylborane, which possesses a benzene ring, is also shown in Fig. 3 (squares). It is clear that this borate compound reduces the ion-exchange capacity for interacting with the resin surface and is superior to boric acid in eluting strength on the grounds that the squares wholly shift downward in comparison to the circles. Thus, the applicability of the capacity gradient technique to the elution of strongly bound species such as condensed phosphates was investigated by using dihydroxyphenylborane. When the mannitol concentration in the eluent was gradually decreased at constant concentrations of the borate compound and sodium hydroxide, the condensed phosphates were eluted at times that depended on their charges. However, the baseline conductance after suppression was reduced along with a decline in the mannitol concentration, because the acid dissociation constant of the mannitol complex may be larger than that of the borate compound. Another problem is that the peak shapes for linear condensed phosphates gradually began to deteriorate. It was thought that the linear phosphates chelated with metals in the tubing, because the IC system used here was not metal-free. To solve these problems, it was helpful to add an infinitesimal amount of EDTA as a masking reagent to the eluent along with the decline in mannitol concentration. Further, sodium hydroxide was used in the gradient to decrease the column capacity. Fig. 4A shows a typical chromatogram with an injection of a mixture of condensed phosphates under these conditions. The baseline drift and the peak shape deterioration were improved and the condensed phosphates, containing trimetaphosphate as a cyclic polymer, having a chain length up to 7 were separated.

On the basis of the above results, this system was applied to the analysis of polyphosphates in real sample. Condensed phosphates are used during the manufacturing processes of various food products to sequester cations, to protect against moisture loss and for other purposes. The results for the separation of cheese extract under the same gradient conditions are shown in Fig. 4B. The separation of orthophosphate from other inorganic anions was performed under isocratic conditions. After the elution of excess TCA as an extractant, condensed phosphates having chain lengths up to 3 were determined by the capacity gradient technique.

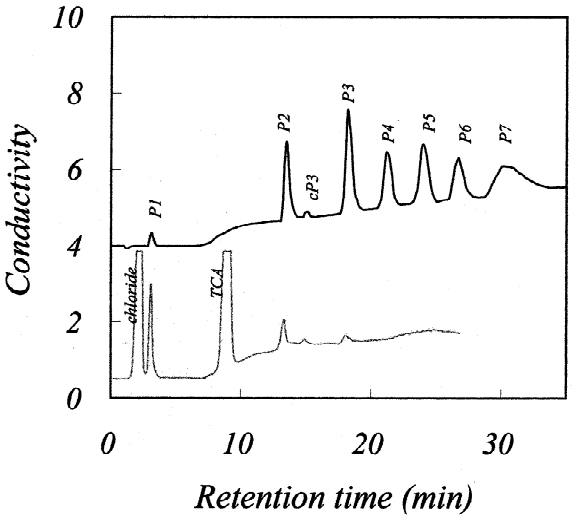

Fig. 4. Capacity gradient ion chromatograms with injections of 5 μl of 0.1% condensed phosphates mixture (solid line) and a cheese extract with TCA (broken line). Chromatographic conditions are given in the text. Peaks: P1=orthophosphate, P2=pyrophosphate, cP3=trimetaphosphate, P3=tripolyphosphate, P4=tetrapolyphosphate, P5–7=higher condensed phosphates with numbers indicating degree of polymerization.

4. Conclusion

Capacity gradient elution based on the formation of an anionic complex between borate and diol compounds was attempted. There are many diol-type hydroxy groups on the resin surface of a Tosoh IC-Anion-PW column, and it was proved that these residues easily formed an anionic complex with borate compounds. The amount of the complex formed could be controlled by adding mannitol to the eluent. Namely, the decrease in mannitol concentration accelerated the formation of the borate–diol complex on the resin surface. This complex formation brought about a decrease in ion-exchange capacity of the column, and strongly bound analyte ions were eluted in order of their charges. This capacity gradient elution technique with a dihydroxyphenylborane–mannitol eluent system made it possible to analyze condensed phosphates in a real food sample.

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