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# Coupling of chromatographic ion-exchange reaction with change-partner reaction Presumed mechanism on the ion–ion interaction of inorganic ions in Sephadex G-15 columns

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## Abstract

A sample solution containing sodium or potassium dihydrogenphosphate or disodium or dipotassium hydrogenphosphate was eluted from a Sephadex G-15 column with either sodium or potassium chloride solution in various sample–eluent systems, and every one of the four kinds of ions employed was determined in the eluate. Then, the elution profiles showed the phenomenon that a negative peak of chloride ion coexisted in the fractions of a positive peak of phosphate ion, in all sample–eluent systems employed. This phenomenon was assumed to occur by the coupled reaction of change-partner and ion-exchange reactions including ion exclusion. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Ion–ion interactions; Sephadex G-15; Inorganic ions

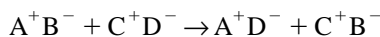
## 1. Introduction

Sephadex G-15 is a dextran gel chromatographic material, capable of fractionating substances with molecular masses less than 1500. However, various side reactions such as solute–gel matrix and solute–solute interactions which alter the elution volume had been observed [1–3].

In our previous experiments [4,5], we eluted

sodium or potassium chloride (NaCl or KCl) solution from a Sephadex G-15 column with sodium or potassium phosphate buffer at pH 7.0 (NaP or KP buffer) in various sample–eluent systems, and determined every one of the four kinds of ions (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, P<sup>-</sup>) employed, in the eluate. Then the two kinds of reactions, ion-exchange and change-partner, were observed in the elution profiles of ions.

The ion-exchange reaction is formulated as



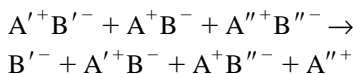
i.e., an ion pair from a sample exchanges the partner ion with an ion pair from the eluent during the elution, producing a cation(from the sample)–P<sup>-</sup> ion pair and a cation (from the eluent)–Cl<sup>-</sup> ion pair. For

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example, when KCl was eluted with NaP buffer,  $K^+P^-$  and  $Na^+Cl^-$  ion pairs were produced [4].

The change-partner reaction is formulated as



with a proviso that  $A'^+$ ,  $A^+$  and  $A''^+$  are the same cation and  $B'^-$ ,  $B^-$  and  $B''^-$  are the same anion, i.e., since the cation,  $Na^+$  or  $K^+$ , tends to be eluted more slowly than the anion,  $P^-$ , the cation of the cation– $P^-$  ion pair is pulled down by the partner  $P^-$ , released from the  $P^-$  gradually, and bound to the other  $P^-$  flowing through later [5].

Another phenomenon we observed was that the  $Na^+P^-$  ion pair is eluted more rapidly than the  $K^+P^-$  ion pair, suggesting that  $Na^+$  is eluted more rapidly than  $K^+$ . The experiments concerning these phenomena were reviewed [6].

This report describes the elution profiles of every one of the four kinds of ions employed when sodium or potassium dihydrogenphosphate or disodium or dipotassium hydrogenphosphate, the constituents of the phosphate buffer, was eluted with either NaCl or KCl solution in various sample–eluent systems. Then, the ion-exchange reaction was also observed. A special feature of the elution profile observed was that a negative peak of  $Cl^-$  coexisted with a positive peak of  $P^-$  in all the sample–eluent systems employed.

A similar phenomenon had been observed previously by Yoza et al. [7] who eluted 0.2 M sodium phosphate ( $Na_3PO_4$ ) with 0.1 M NaCl solution from a Sephadex G-15 column. Deguchi et al. [8] eluted 0.05 M sodium sulfate, oxalate, acetate or fluoride from a Sephadex G-15 column with 0.1 M NaCl solution, and observed the phenomenon that a negative peak of  $Cl^-$  was produced in the fractions of a positive peak of anion from the sample. They assumed that the  $Cl^-$  was excluded by the anion from the sample to the later fractions. However, since they did not determine the cation, they could not observe all of the ion–ion interactions during the elution.

We observed the elution profiles of every one of the four kinds of ions employed and assumed that not only the exclusion of  $Cl^-$  by  $P^-$  but also coupled ion-exchange and change-partner reactions were involved in the phenomenon.

## 2. Experimental

### 2.1. Chemicals

Sodium chloride (NaCl), potassium chloride (KCl), sodium dihydrogenphosphate ( $NaH_2PO_4 \cdot 2H_2O$ ), disodium hydrogenphosphate ( $Na_2HPO_4 \cdot 12H_2O$ ), potassium dihydrogenphosphate ( $KH_2PO_4$ ) and dipotassium hydrogenphosphate ( $K_2HPO_4$ ) were of analytical-reagent grade from Wako (Osaka, Japan).

### 2.2. Samples and eluents

The sample solution was 1 M  $KH_2PO_4$  (KP), 1 M  $K_2HPO_4$  ( $K_2P$ ), 1 M  $NaH_2PO_4 \cdot 2H_2O$  (NaP) or 0.125 M  $Na_2HPO_4 \cdot 12H_2O$  ( $Na_2P$ ) dissolved in the eluent. Since  $Na_2P$  was not completely dissolved in the eluent at a concentration of 0.25 M or more at 4°C, but was dissolved completely at 0.125 M, we used 0.125 M  $Na_2P$  as a sample solution.

The eluent was either 0.05 M NaCl or 0.05 M KCl solution.

Five sample–eluent systems were employed; a homocationic heteroanionic system (referred to homocationic system in this paper) of NaP:NaCl and four heterocationic heteroanionic systems (referred to heterocationic systems in this paper) of KP:NaCl,  $K_2P$ :NaCl, NaP:KCl and  $Na_2P$ :KCl.

### 2.3. Procedure

Sephadex G-15 (Pharmacia, Uppsala, Sweden; dry particle diameter, 40–120  $\mu m$ ) was packed according to a standard procedure in a glass column (Excel type SE-1000; 1000×19 mm I.D.; bed height, 90 cm; porous polystyrene support). In the preparation of the column, care was taken to prevent irregularity of the gel surface as this causes the appearance of a small artificial peak in the earliest part of the elution profile. A peristaltic pump (LKB Instruments, Rockville, USA; gear ratio 3:250) was inserted between the eluent reservoir and the top of the column to maintain a constant flow-rate (12 ml/h).

As phosphate ion ( $P^-$ ) binds to the gel tightly and scarcely exchanges with  $P^-$  in the eluent under the test conditions [9], before use we equilibrated the gel with 0.025 M sodium phosphate buffer (pH 7.0) to

prevent adsorption of  $P^-$  from the sample to the gel. We then washed and equilibrated the gel with the eluent. A sample of 0.6 ml of solution was applied to the top of the column. The eluate was continuously monitored with a Refractometer Model SF1107 (Mitsumi Scientific, Tokyo, Japan) and collected in 10-min fractions using a LKB7000 Ultrarac fraction collector (LKB Instruments).

Elutions were performed in a cold room at 4°C to avoid change of the elution behavior of ions by temperature shift.

#### 2.4. Quantitation of ions

The amounts of sodium ( $Na^+$ ) and potassium ions ( $K^+$ ) were determined in a Corning Model 480 flame photometer (Corning Medical, Sudbury, UK), and chloride ions ( $Cl^-$ ) were measured in a Corning Model 925 chloride analyzer (Corning Medical). The phosphate ion, irrespective of dihydrogen or monohydrogen, was determined by the method of Fiske and Subbarow [10].

#### 2.5. Drawing of the figures

In order to see the ion–ion interactions between the ions from the sample and the eluent more easily in a figure, the zero level of the concentration of ions from the sample was adjusted to 50  $\mu\text{mol}$  per ml of ions from the eluent. An exception was Fig. 2B ( $Na_2P:KCl$  system) in which one scale was used for both ions from the sample and the eluent, because of the very low concentration of the sample salt.

### 3. Results

#### 3.1. Elution profiles of ions in heterocationic systems

##### 3.1.1. $KP:NaCl$ system (Fig. 1A)

When 1 M  $KH_2PO_4$  solution was eluted with 0.05 M NaCl solution, the elution profiles of ions showed a positive peak of  $Na^+P^-$  ion pair in the early fractions, which was followed by a shoulder and a negative peak of  $Na^+Cl^-$  ion pair and a positive peak of  $K^+Cl^-$  ion pair sequentially. Another nega-

tive peak of  $Cl^-$  coexisted with a positive peak of  $P^-$  in the early fraction.

A noticeable feature of the elution profile was that the positive peak of  $P^-$  was higher and more expanded to the earlier fractions than the positive peak of  $Na^+$ . This phenomenon was observed in all sample–eluent systems employed.

The phenomenon that the positive peaks of  $Na^+P^-$  and  $K^+Cl^-$  ion pairs were produced clearly indicated that an ion-exchange reaction occurred between the ion pairs from the sample and the eluent during the elution. The phenomenon that the positive peak of  $K^+Cl^-$  ion pair followed a negative peak of  $Na^+Cl^-$  ion pair from the eluent, indicating that the  $K^+Cl^-$  ion pair was eluted more slowly than the  $Na^+Cl^-$  ion pair. This phenomenon also suggests that  $K^+$  is eluted more slowly than  $Na^+$  as was assumed in our previous report [4].

##### 3.1.2. $K_2P:NaCl$ system (Fig. 1B)

When 1 M  $K_2HPO_4$  solution was eluted with 0.05 M NaCl solution, a similar elution profile was observed.

However, contrary to the elution profiles observed in the  $KP:NaCl$  system,  $Na^+$  decreased more rapidly than  $Cl^-$  in the fractions of the second negative peak of  $Cl^-$ . This phenomenon might be explained by assuming that ion-exchange reaction in  $K_2P:NaCl$  system produced two-times more  $K^+Cl^-$  ion pair than the  $KP:NaCl$  system, and the  $K^+Cl^-$  ion pair expanded to the earlier fractions and overlapped with the  $Na^+Cl^-$  ion pair, resulting in excess  $Cl^-$  over  $Na^+$  in the overlapping fractions.

##### 3.1.3. $NaP:KCl$ system (Fig. 2A)

When 1 M  $NaH_2PO_4$  solution was eluted with 0.05 M KCl solution, positive peaks of  $K^+P^-$  and  $Na^+Cl^-$  ion pairs were produced in early and late fractions, respectively, again indicating that an ion-exchange reaction occurred between the ion pairs from the sample and the eluent.

A negative peak of  $Cl^-$  coexisted with a positive peak of  $P^-$ . However, contrary to the results obtained in the former systems (Fig. 2A and B), the peaks of  $Na^+$  and  $Cl^-$  trailed to the earlier fractions and a small  $Na^+$  peak was produced in the latter half of the positive peak of  $P^-$ , and a small positive peak of  $Cl^-$  following the positive peak of  $P^-$  was

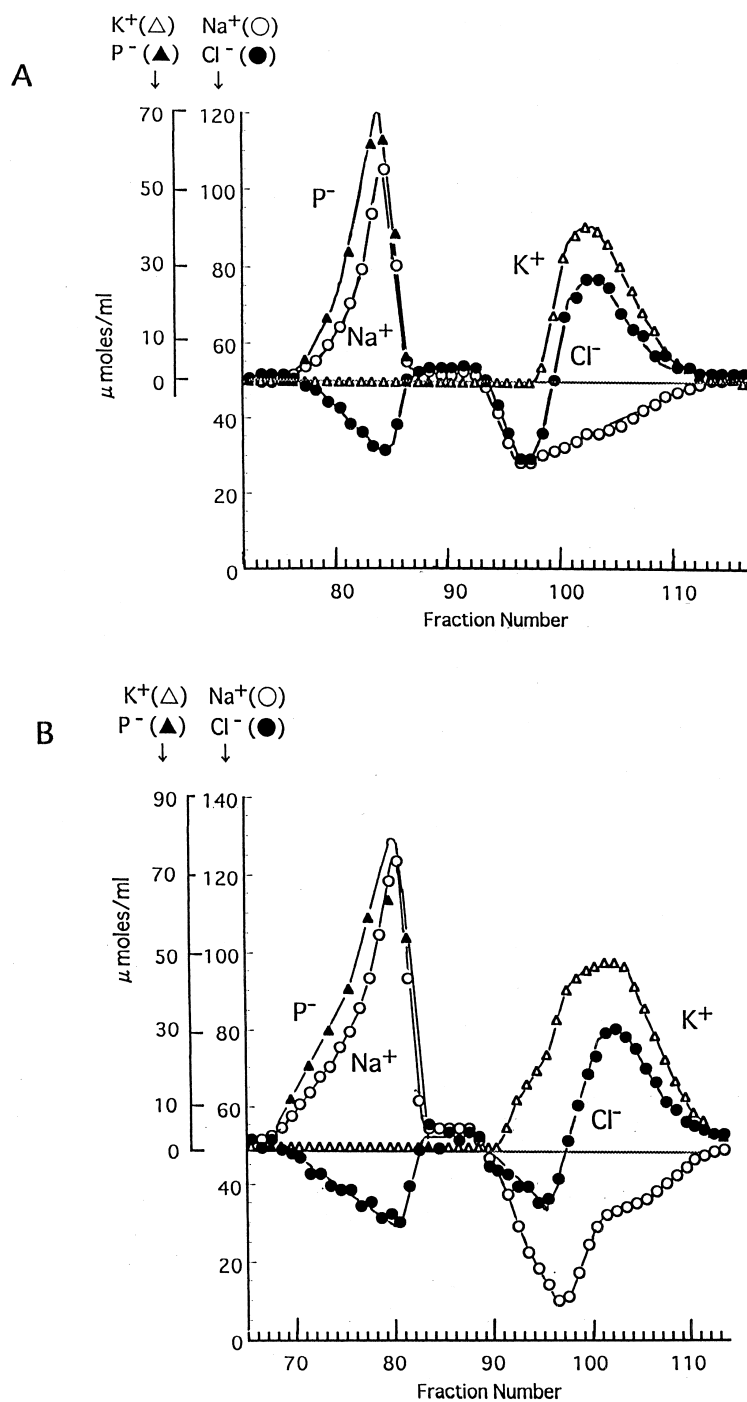


Fig. 1. Elution profiles of ions when 1 M  $KH_2PO_4$  (A) or 1 M  $K_2HPO_4$  (B) was eluted with 0.05 M NaCl solution.  $Na^+$  ( $\circ$ ),  $Cl^-$  ( $\bullet$ ),  $K^+$  ( $\Delta$ ),  $P^-$  ( $\blacktriangle$ ).

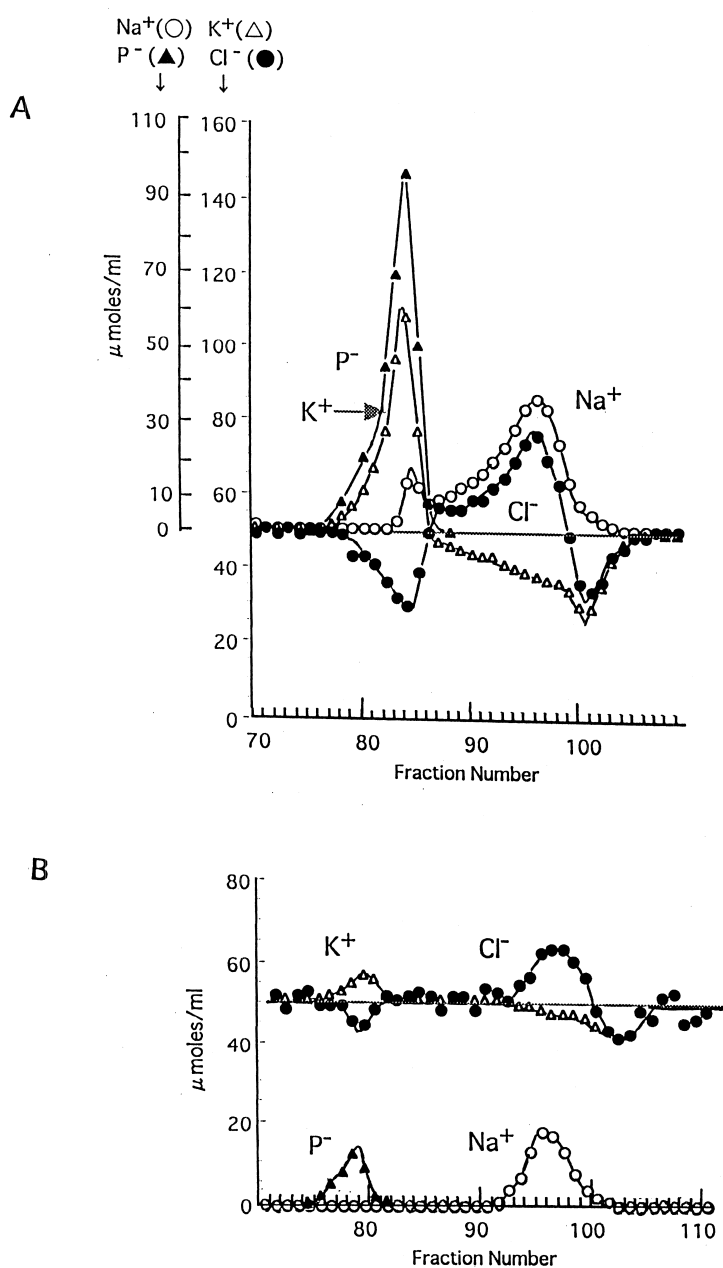


Fig. 2. Elution profiles of ions when 1 M NaH<sub>2</sub>PO<sub>4</sub> (A) or 0.125 M Na<sub>2</sub>HPO<sub>4</sub> (B) was eluted with 0.05 M KCl solution. Na<sup>+</sup> (○), Cl<sup>-</sup> (●), K<sup>+</sup> (△), P<sup>-</sup> (▲).

produced. The small Na<sup>+</sup> peak might be the Na<sup>+</sup>P<sup>-</sup> ion pair from the sample which had not yet been subjected to the ion-exchange reaction, and the small Cl<sup>-</sup> peak might be formed by overlapping the

Na<sup>+</sup>Cl<sup>-</sup> ion pair with the K<sup>+</sup>Cl<sup>-</sup> ion pair from the eluent.

The positive peak of Na<sup>+</sup>Cl<sup>-</sup> ion pair was followed by a negative peak of K<sup>+</sup>Cl<sup>-</sup> ion pair, again

indicating that the  $\text{Na}^+\text{Cl}^-$  ion pair was eluted more rapidly than the  $\text{K}^+\text{Cl}^-$  ion pair.

### 3.1.4. $\text{Na}_2\text{P}:\text{KCl}$ system (Fig. 2B)

When 0.125 M  $\text{Na}_2\text{HPO}_4$  solution was eluted with 0.05 M KCl solution, all the peaks produced were small and separated from each other, because of the low concentration of the sample salt. In spite of such a low concentration of the sample salt, the  $\text{Cl}^-$  negative peak was observed in the fractions of a positive peak of  $\text{P}^-$ .

### 3.2. Elution profiles of ions in a homocationic system (Fig. 3)

When 1 M  $\text{NaH}_2\text{PO}_4$  solution was eluted with 0.05 M NaCl solution (NaP:NaCl system), the elution profiles of ions showed a positive peak of  $\text{Na}^+\text{P}^-$  ion pair in the early fractions, and a low but broad positive peak of the  $\text{Na}^+\text{Cl}^-$  ion pair in the late fractions. A  $\text{Cl}^-$  negative peak coexisted with the positive peak of  $\text{P}^-$ . As the  $\text{Na}^+\text{Cl}^-$  ion pair formed by ion-exchange reaction is the same as that of the eluent, the second negative peak of  $\text{Cl}^-$  was not formed.

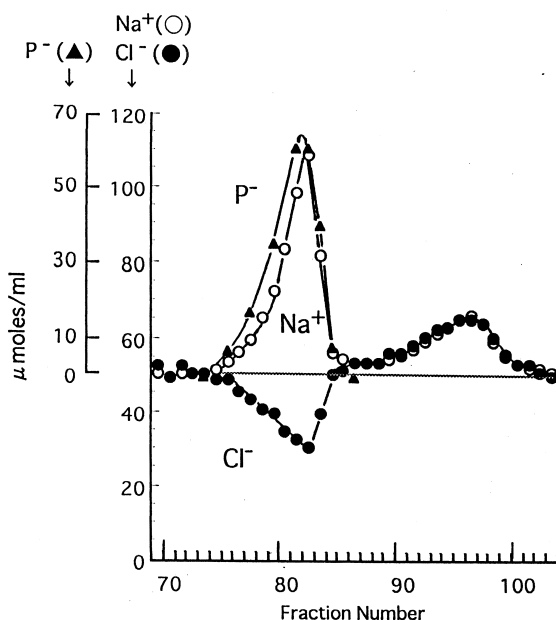


Fig. 3. Elution profiles of ions when 1 M  $\text{NaH}_2\text{PO}_4$  was eluted with 0.05 M NaCl solution.  $\text{Na}^+$  ( $\circ$ ),  $\text{Cl}^-$  ( $\bullet$ ),  $\text{K}^+$  ( $\triangle$ ),  $\text{P}^-$  ( $\blacktriangle$ ).

The mechanism why the positive peak of  $\text{Na}^+\text{Cl}^-$  ion pair was produced is as follows. The high concentration of  $\text{Na}^+$  from the sample collects many  $\text{Cl}^-$  ions from the  $\text{Na}^+\text{Cl}^-$  ion pair of the eluent by ion-exchange reaction, producing a higher concentration of  $\text{Na}^+\text{Cl}^-$  ion pair than in the eluent, which was distributed to many later fractions as the  $\text{P}^-$  proceeds.

## 4. Discussion

This paper describes the elution profiles of every ions employed ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{P}^-$ ) when a solution of  $\text{NaH}_2\text{PO}_4$  (NaP),  $\text{KH}_2\text{PO}_4$  (KP),  $\text{Na}_2\text{HPO}_4$  ( $\text{Na}_2\text{P}$ ) or  $\text{K}_2\text{HPO}_4$  ( $\text{K}_2\text{P}$ ) was eluted from a Sephadex G-15 column with either NaCl or KCl solution in various sample–eluent systems. Then, various elution profiles of ions were observed depending on the system employed. However, a common feature of the elution profile to all sample–eluent systems employed was that a negative peak of  $\text{Cl}^-$  coexisted with a positive peak of  $\text{P}^-$ . This phenomenon has been ascribed to the exclusion of  $\text{Cl}^-$  by  $\text{P}^-$  [7,8]. However, we assumed that not only exclusion, but also coupled ion-exchange and change-partner reactions were involved in this phenomenon.

### 4.1. Hypothesis

We would like to take the KP:NaCl system (Fig. 1A) as an example for explaining our hypothesis on the ion–ion interactions during elution in a column.

When 1 M  $\text{KH}_2\text{PO}_4$  solution was applied to the top of a Sephadex G-15 column, the concentration distribution of the  $\text{K}^+\text{P}^-$  ion pair would be symmetrically positive. Therefore, in the early half of the  $\text{P}^-$  peak, the concentration of the  $\text{K}^+\text{P}^-$  ion pair increases to the later fractions, and in the late half of the  $\text{P}^-$  peak, decreases to the later fractions. As soon as the  $\text{K}^+\text{P}^-$  ion pair begins to be eluted, change-partner reaction occurs. In the early half of the  $\text{P}^-$  peak, the amount of the free  $\text{P}^-$  produced by the change-partner reaction is excess over the  $\text{K}^+$  which is released from the  $\text{K}^+\text{P}^-$  ion pair in the earlier fractions. Therefore, the excess free  $\text{P}^-$  pairs with  $\text{Na}^+$  of the  $\text{Na}^+\text{Cl}^-$  ion pair of the eluent,

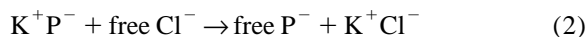
resulting in producing both the  $\text{Na}^+\text{P}^-$  ion pair and free  $\text{Cl}^-$ . This reaction is formulated as



This is a half ion-exchange reaction.

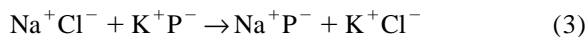
As the free  $\text{Cl}^-$  thus produced is not only eluted more slowly than  $\text{P}^-$  but also excluded by the free  $\text{P}^-$  to the later fractions, the free  $\text{Cl}^-$  enters into the latter half of the  $\text{P}^-$  peak promptly.

In the late half of the  $\text{P}^-$  peak, the  $\text{K}^+$  which is released from  $\text{K}^+\text{P}^-$  ion pair by change-partner reaction is excess over the free  $\text{P}^-$  produced in the later fractions. Therefore, the excess free  $\text{K}^+$  pairs with the free  $\text{Cl}^-$  which has been produced in the early half of the  $\text{P}^-$  peak. This reaction is formulated as



This is also a half ion-exchange reaction.

The overall reaction, i.e., Eqs. (1+2), is formulated as

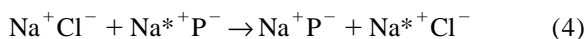


This equation is that of the ion-exchange reaction, i.e., the  $\text{K}^+\text{P}^-$  ion pair from the sample and  $\text{Na}^+\text{Cl}^-$  ion pair of the eluent exchanges their partner ion, producing the  $\text{Na}^+$  (from the eluent)– $\text{P}^-$  and  $\text{K}^+$  (from the sample)– $\text{Cl}^-$  ion pairs. Therefore, the ion-exchange reaction is accomplished by coupling the change-partner reaction and the two half ion-exchange reactions.

The amount of the free  $\text{Cl}^-$  produced in the early half of the  $\text{P}^-$  peak is approximately equal to the amount of the free  $\text{K}^+$  produced in the late half of the  $\text{P}^-$  peak and the ion-exchange reaction proceeds smoothly. The  $\text{K}^+\text{Cl}^-$  ion pair is eluted more slowly than  $\text{Na}^+\text{Cl}^-$  ion pair of the eluent and is removed to the later fractions. Thus the second negative peak of  $\text{Cl}^-$  is produced, which is followed by a positive peak of  $\text{K}^+\text{Cl}^-$  ion pair.

The ion-exchange reaction continues until  $\text{K}^+$  of all  $\text{K}^+\text{P}^-$  ion pair is replaced by  $\text{Na}^+$ . Then, the free  $\text{Cl}^-$  produced in the early half of the  $\text{P}^-$  peak pairs with the free  $\text{Na}^+$  produced in the late half of the  $\text{P}^-$  peak. Thus,  $\text{Na}^+$  (from the  $\text{Na}^+\text{P}^-$  ion pair)– $\text{Cl}^-$  ion pair, which is eluted with the  $\text{Na}^+\text{Cl}^-$  ion pair of the

eluent. A shoulder of  $\text{Na}^+\text{Cl}^-$  ion pair is thus produced. This reaction is formulated as



This is an equation of the ion-exchange reaction in homocationic system. These reactions are operated during elution removing the  $\text{Cl}^-$  from fractions of the positive peak of  $\text{P}^-$  to the later fractions continuously. This is the mechanism which allows a negative peak of  $\text{Cl}^-$  to coexist with the positive peak of  $\text{P}^-$ .

#### 4.2. Quantitative analysis

The elution profiles of ions observed in the KP:NaCl system (Fig. 1A) were quantitatively analyzed as follows.

$\text{Na}^+$  (Fig. 4A): The amount of  $\text{Na}^+$  in the positive peak is calculated to be equal to the amount of  $\text{Na}^+$  which was lost from the fractions of the negative peak of  $\text{Na}^+$ . Thus the  $\text{Na}^+$  was concentrated in the fractions of the positive peak of  $\text{P}^-$ .

$\text{Cl}^-$  (Fig. 4B): The amount of  $\text{Cl}^-$  in a positive peak is calculated to be sum of the amount of  $\text{Cl}^-$  which is lost from the fractions of the two negative peaks of  $\text{Cl}^-$ , indicating that  $\text{Cl}^-$  in the positive peak is derived from the fractions of the two negative peaks.

$\text{K}^+$  (Fig. 4C): The amount of  $\text{K}^+$  in the positive peak is calculated to be equal to the amount of excess  $\text{Cl}^-$  over  $\text{Na}^+$  in each fraction of the positive peak of  $\text{K}^+$ . This result shows that the  $\text{K}^+$  pairs with  $\text{Cl}^-$  in the fractions.

$\text{P}^-$  (Fig. 4D): The  $\text{P}^-$  expanded to the earlier fractions than  $\text{Na}^+$  in the peak fractions might be free  $\text{P}^-$ . The amount of the free  $\text{P}^-$  is calculated to be almost identical with the amount of  $\text{Cl}^-$  which was lost from the fractions of the early negative peak of  $\text{Cl}^-$ , supporting the idea that the free  $\text{P}^-$  excludes the free  $\text{Cl}^-$  to the later fractions.

#### 4.3. The other systems

The special features of the elution profiles in each system other than the KP:NaCl system were discussed in the Results section. The phenomenon that a negative peak of  $\text{Cl}^-$  coexisted in the fractions of the

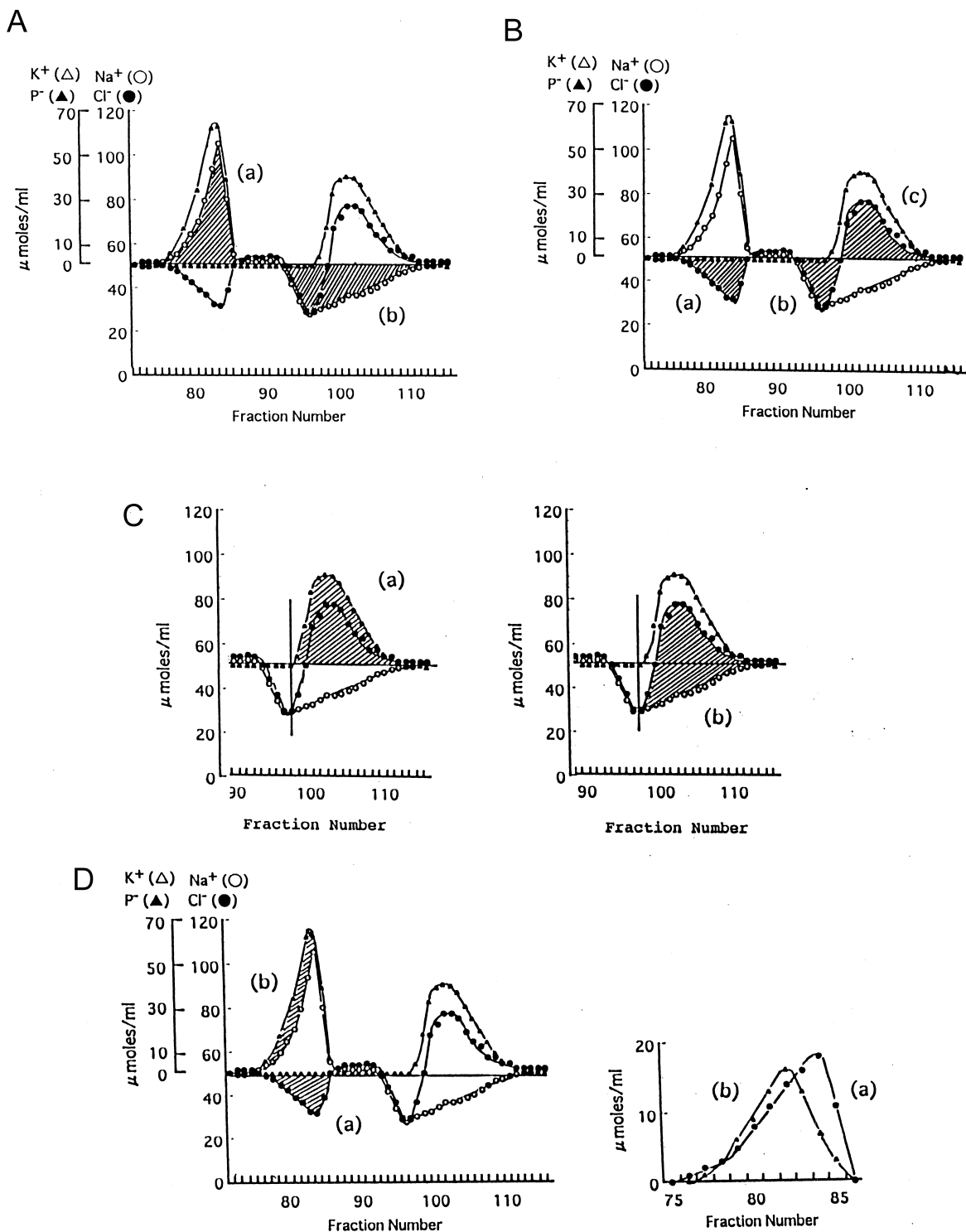


Fig. 4. Quantitative analysis of the elution profiles of ions. (A)  $Na^+$ : [ $a=b$ ], (B)  $Cl^-$ : [ $a+b=c$ ], (C)  $a(K^+)=b(Cl^- - Na^+)$ , (D)  $b(P^- - Na^+) = a(-Cl^-)$ .



