Development of an Ion Stacking Method by Aqueous Partition Chromatography with a Hydrophilic Polymer Gel Column

(親水性高分子ゲルカラムを用いた水系分配クロマトグラフィーによるイオン濃縮法の開発）

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Chapter 1

General Introduction

1.1 Preface

Enhancing the sensitivity of trace amounts of ions in the accompanying large amounts of components is a common problem prior to analysis. Although a lot of spectrophotometric [1-9] and electrochemical [10-12] methods are used for enrichment of inorganic and organic anions in aqueous samples, ion chromatography has gained immense attention and is often treated as the most acceptable technique for monitoring trace amounts of inorganic ions in water samples [13-17]. Besides, Liquid-liquid extraction (LLE) [18, 19] and solid-phase extraction (SPE) [19, 20] are widely used as techniques for separation and preconcentration of both organic and inorganic analytes for various applications.

Despite of well performance of the aforementioned methods, some bottlenecks are frequently observed. For example, spectrometric and electrochemical methods fail to detect trace ions in solutions containing large excess of foreign ions due to low sensitivity. With the SPE technique, the analyte ions are stripped from the sample solution onto an SPE concentrator column and then eluted from the column with a suitable eluent to the analytical column. The use of organic solvents and/or toxic reagents for extraction or elution of the analyte ions in SPE methods make them expensive, tedious, and time consuming. Furthermore, to ensure accurate and precise determination of trace ion, analyte with high loading and elution efficiency are required. Due to these problems, there are very few, if any successful methods that can be used for the enrichment of ionic solutes especially for inorganic anions in water samples. Therefore, On-column concentration may be one of the methods which can minimize the loss of the analytes in the preconcentration process. Accordingly, the author tried to develop a versatile method for enrichment of ionic solutes in aqueous solution using on-column concentration. Some conventional on-column concentration methods are described below in brief.

Ion exchange is widely used for the separation and enrichment of ionic solutes. In ion exchange mechanism, ionic solutes are separated on a packing material carrying fixed charges by ion exchange reaction, in which ions in the stationary packing material and in the solution are exchanged in equivalent amount based on the difference in affinity of different ions for the fixed ionic groups on the packing materials [21-23]. The separation depends on the selectivity of ion exchanger, type of electrolyte in the mobile phase, pH, ionic strength, and complex forming ability. The successful separation of any kind of ionic solutes such as amino acid, inorganic and organic ions by ion exchange mechanism has been reported. Ion exclusion is one type of ion exchange mechanism also used for the separation of ions, where ions of the same charges that of functional group on the packing material are excluded due to the electrostatic repulsion.

Ion-pair chromatography is used as a typical separation method for the separation of ionic solutes with a nonpolar hydrophobic surface using mobile phase additives or ion pairing reagents. The ion pairing reagents contain some functional groups adsorb or distribute to the nonpolar stationary phase and converts the nonpolar stationary phase to an ion exchanger which can exchange ions of opposite charge and excludes ions of the same charge. The retention selectivity can be manipulated by the concentration of ion pairing regents and the percentage of organic solvent in the mobile phase [24]. However, it takes a long time to attain the adsorption equilibrium of the ion pairing reagents on the packing material.

1.2 The background/Origin of the present research

It has been reported that non-ionic or electrically neutral materials are capable of separating not only organic ions but also simple inorganic ions in an aqueous solution without any additives such as ionic surfactants for ion-pair formation. Separation of metal ions and simple anions by various types of hydrophilic polymer gels, *i.e.*, cross-linked dextran, polyacrylamide gels, etc. has been studied since 1960s [25-41]. It has also been shown in recent years that even hydrophobic materials such as polystyrene resin and octadecyl bonded silica [42-52] exhibits selective ion separation.

The water-soluble polymer gel is originally used as filler for water-based size exclusion chromatography (SEC). SEC is a technique of separation depending on the molecular size or molecular weight of the compounds to be separated. As well as the separation, an interesting finding of ion enrichment in aqueous solution has been reported.

Recently, Davankov *et al.* developed a simple, unique and cost effective enrichment method for enrichment of ionic solutes in aqueous solutions [42-44]. They developed a uniform nanoporous hypercrosslinked polystyrene packing material and showed that an enhancement of the concentration of component ion takes place when a mixture of two different electrolytes is injected and eluted with pure water. These separation or enrichment behaviors of inorganic ions exerted by the porous materials are attempted to explain in terms of size-exclusion mechanism because the retention order corresponds to the radii of the hydrated ions. In brief, this explanation is based on the fact that ions with larger hydrated radii usually show smaller retention volumes. For example, when the mixed solution enters the column, Ca^{2+} elutes at the front end of the sample zone because it has a large hydrated ionic radius and does not enter into the pores of the polymer and concentrated at the outer surface of the gel material. On contrary, H^+ elutes at the rear end of the sample zone and concentrates inside of the pores of the gel material by the electrostatic repulsion effect of Ca^{2+} existing outside the pores. The stationary polystyrene resin was assumed to function as an inert material and the solvent (water) occupies the space inside the pores. However, this size-exclusion mechanism cannot explain fully the phenomena of ion concentration in polymer gel column.

If dry polymer columns are used, an enrichment of solute compounds that have large molecular sizes may occur by injection of the sample solution into the column because water can be sorbed into the gel, while large solute molecules may not penetrate into the polymer network. However, the increase in concentration of solute molecules does not take place when the sample solution comes into contact with water incorporated polymer gels even though the solute molecules are excluded from the gel, since the interior of the polymer gels has already been filled with water. Therefore, it is impossible to account for enrichment of ions on water swollen polymer gel columns in terms of size-exclusion mechanism. In fact, the polymer gel columns equilibrated with water were used for all the studies described above.

In addition, it has been reported that water exhibits anomalous physiochemical properties at hydrophobic and hydrophilic gel surface due to the interaction of water molecules with the polymer. Tamai *et al.* investigated the structure and dynamics of water molecules in hydrophilic polymer gels by molecular dynamic simulation. The water-water hydrogen bonds in polyvinyl alcohol, polyvinyl methyl ether and poly (N-isopropylacrylamide) gels were stabilized and numbers of hydrogen bonds present in the water swollen polymers were estimated [54]. Static properties of hydrogen-bonding networks were analyzed to evaluate the interplay between water and polymer. The perturbed structure of water at the hydrophobic and hydrophilic interface caused by stretching of hydrogen bond in the vicinity of polymer has also been detected by NMR [55], DSC [55] and other techniques [56-58].

Several studies enumerated that the sorbed water has different state than ordinary bulk water and is categorized in three states by different techniques [59-63]. DSC is the most frequently used method for investigation of water states in polymer gel. The amount of water which melts at a temperature of 273K and same as ordinary bulk water is defined as freezing water while the freezable bound water is regarded to water which melts at a temperature lower than 273K. The non-freezable water is termed to that, which requires a very lower temperature than 273K for melting or impossible to observe crystallization exotherms. The freezable bound and non-freezable water are formed by the interaction of water molecules with the polymer. In brief, the physiochemical properties of sorbed water are different from bulk water. Although the content of sorbed water in hydrophilic gel is larger than that of hydrophobic gel, and the water absorbed in hydrophilic gel materials is not physically separated from gel or the gel matrix forms a mixed solution phase of water and gel, it has been reported that hydrophobic surface can contain such a type of structure perturbed interfacial water which has been investigated through the observation of stretching of hydrogen bond present in water molecules [46, 64-66]. The presence of different states of interfacial water at a hydrophobic surface is also brought forth in some studies [46]. As a matter of fact, hydrogen bonds present in water altered by the interaction of water molecules with the polymer matrix and the physiochemical properties of the water in the vicinity of the polymer chains becomes different from the bulk water.

In addition, Shibukawa *et al.* demonstrated the relationship of these three states of water with the separation selectivity of polymer gels, using several hydrophilic polymer gels (dextran, polyacrylamide gel, polyethylene glycol , polyvinyl alcohol) [59-63] with different degrees of crosslinking by measuring the distribution coefficients of various organic compounds eluting with aqueous mobile phase. The amount of stationary phase water which exhibit different affinity to solute than bulk water was calculated by measuring the mobile phase volume determined by a liquid chromatographic method based on the effect of mobile phase electrolyte on the retention of ionic solute [52, 53]. They pointed out that in many hydrophilic polymer gels, non-freezable water and freezable water function as the stationary phase, but in some gels only nonfreezing water functions as the stationary phase. According to their suggestions, the stationary phase can be assumed as a mixed solution of polymer matrix and water. They concluded that the amount of water in a polymer gel that exhibits different affinity to solute compounds from the bulk water can functions as the stationary phase that can be estimated in any polymer surface by their liquid chromatographic method. However, in order to investigate the separation process, it is necessary to evaluate the research developed to date to investigate the actual solute retention mechanism during elution through a polymer gel column and assumption of the stationary phase that acts as separation medium. There are several models those tried to explain the separation process based on adsorption or molecular sieve effect.

If only the molecular sieve effect contributes to the retention of solute, the retention volume V_R of the solute should not exceed the total liquid phase volume V_t in the column, but in practice a holding volume greater than V_t is obtained. It is believed that adsorption and distribution dominantly act on retention rather than molecular sieve effect (secondary effect).Three different phenomena of solute retention can be considered.

1. If the state of liquid inside the pore of the gel material and incorporated liquid state in the vicinity of gel itself is same and molecular sieve effect controls the adsorption [49] (Figure 1.9 a), then K_{ads} will be the partition coefficient of solute based on adsorption, V_0 is the liquid phase volume outside the filler particle, and $\boldsymbol{V_i}$ is the liquid phase volume inside the particle.

2. If it is assumed that state of the liquid phase inside the pore of the packing material becomes different by the interaction with the packing material than bulk liquid (Figure 1.1b), the distribution is governed by the molecular sieve effect or size exclusion mechanism [50], then K_D is the partition coefficient of the solute between the bulk liquid and liquid phase inside the pore of the packing material, V_0 is the liquid phase volume outside the packing material, and V_i is the liquid phase volume inside the pores of the packing materials.

3. In 1981, Shibukawa *et al.* revealed that gel phase consisting with sorbed water functions as stationary phase in the separation process (Figure 1.1 c) [34]. Where K_D is the partition coefficient of the solute where steric exclusion and partition governs the separation that occurs between the outside the gel particle/mobile phase and the gel phase containing the absorbd solvent. V_X is the volume of the gel phase which can be determined by the sum of the volume of the base polymer itself of the gel and the liquid phase volume inside the gel particle.

Later in 1986, Shibukawa group interpreted the chemical separation of ionic compounds not only on water incorporated hydrophilic polymer gel columns but also on the columns packed with hydrophobic materials on the basis of a stoichiometric ion partition model [45, 52, 53]. They illustrated that the distribution of ions on hydrophilic gel takes place with the partition between the bulk water and gel phase consisting with interfacial water, while on hydrophobic gel the distribution of ions takes place by the partition between bulk water and interfacial water formed on the surface. However, the partition model has not yet been applied to the explanation of the ion enrichment phenomena in liquid chromatographic technique with nonionic hydrophobic and ionic hydrophilic polymer gel columns.

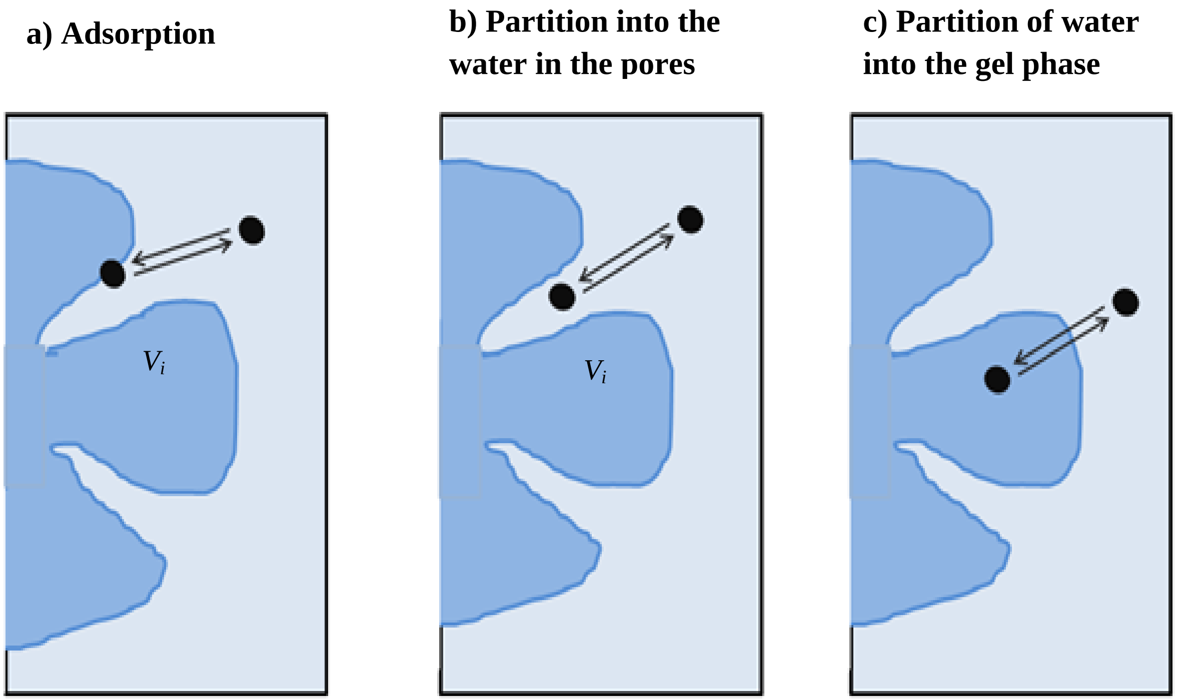

Figure 1.1 Schematic representations of the partition models of ion in water incorporated gel

1.3 Effect of electrolytes in the distribution process

The separation and concentration behavior of inorganic ions that reported by Davankov group was conducted with the polymer material containing no functional group. They added one more electrolyte for separation and enrichment of ions. But it has been reported that it is possible to separate small inorganic ions on dextran and polyacrylamide gels without the help of additional electrolyte using pure water as eluent. Saunders *et al.* have found that it is possible to separate inorganic ions using pure water as an eluant with a polyacrylamide gel as filler [25]. It has been reported that the distribution coefficient depends on the concentration of sample ion when using water as eluant. It is believed that many of the gel materials contain some fixed anionic groups which repulse anions from the gel material when eluted with water or eluent of low concentration and functions as ion exchanger for cations and excludes anions [26, 29]. In such case, in order to maintain the electroneutrality of solution, the constituents of sample cation and anions elute together. Thus, the distribution coefficient of sample ion depends not only on the component cation but also component anion of a salt solution. Although eluent of high ionic strength can suppress this ion exclusion effect exerted from the gel material, still distribution coefficient of analyte ions can be affected by the type of electrolytes and amount of sample solution or eluent. As a matter of fact, during elution in such type of gel, the presence of eluent electrolyte ensures the frequent movement of a sample ion if it is a constituent of eluent salt.

In addition, the elution profile/peak shape is also affected when elution is carried out on hydrophilic gel carrying some fixed charges. In such system, the top of the peaks become flat at low sample concentration while at high concentration of sample ion, the peaks exhibit broadened leading edge with a sharp trailing edge [26]. The elution curve of sample ions shifts to a higher elution volume with increasing sample concentration, thus the distribution coefficient increases with increasing sample concentration. The elutions of peaks start from the void volume due to the effective exclusion effect of fixed functional group.

Shibukawa group tried to explain the elution profile in hydrophilic polymer gel and established a relationship of chromatographic behavior and sample concentration in terms of partition isotherm [26] with the partition between the bulk water and the water incorporated polymer matrices [45, 57]. According to their explanation, if a system is considered where the eluent concentration is high enough to suppress the ion exclusion effect and the sample ion is S^{m+} and eluant co-ion is Y^{p+} in ion exchange process, K_B is the distribution coefficient that determines the sample concentration dependence of the elution behavior of sample ion. Then the following relationships obtained from the partition which can be given below.

When sample concentration is increased, the distribution coefficient of sample ion decreases and more skewed shaped peak is obtained with sharp leading edge when concentration of ionic solute is placed in X axis and concentration in gel phase is placed in Y axis of a partition isotherm, then the relationship from the curve can be expressed by $K_B > 1$.Oppositely, when the relationship is $K_B < 1$, the distribution coefficient of sample ion decreases and the elution profile becomes more skewed with a sharp trailing side with increasing sample concentration. Therefore, the distribution coefficient will be constant and elution profile becomes symmetrical regardless of sample concentration when $K_B = 1$.

Generally it is observed that the addition of electrolyte to the mobile phase improves the peak shape and sharp peak can be obtained if the concentration of the added electrolyte is high enough to suppress the ion exclusion effect completely. Moreover, the retention behavior of an analyte ion is affected by the electrolyte present in mobile phase. According to several reports, the retention volume of analyte become larger when the mobile phase contains weakly retained co-ion electrolyte in the mobile phase, while retention volumes becomes smaller during elution with strongly retained electrolyte [34-36, 53]. In fact, the distribution coefficient of ions depends on the type of co-ion and counter-ion exists in the eluent or sample. The effect of counter-ion and co-ion on the distribution coefficient of analyte ion will be discussed in details in the next chapter by a stoitiometric ion partition model which is based on distribution process of ion in water incorporated gel.

1.4 Goal of this study

Taking into account the observations described above, the author anticipated that the enhancement of concentrations of the component ions in a solution during migration through nonionic polymer columns can be explained by the partition between the bulk water and the stationary phase water incorporated in the polymer, and this ion enrichment effect is not limited to nanoporous material but should be observed for any materials that incorporate water in their matrices. It will be shown in this thesis that the ion enrichment in liquid chromatographic processes can indeed be interpreted in terms of the ion partition model.

1.5 Summary of the thesis

The 2nd chapter shows an explanation of the ion partition model and the mathematical expressions. The third chapter shows the utilization of the model for the explanation of enrichment mechanism on the non-ionic hydrophobic silica and ionic hydroxylated methacrylate surfaces. The fourth chapter delivers the information of the validation of this method into real sample and reagent grade salts. Reference to tables, figures and literature apply only to those references contained within that section. At last, the Chapter 5 contains general conclusions and the future perspectives of the study.

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Chapter 2

THEORETICAL

It can be considered that the strong electrolyte is completely dissociated in the aqueous solution. In a partition chromatographic system where the association of a cation and an anion is negligible in both the mobile and stationary phases and fixed ionic groups are absent in the stationary phase, a cation C^{p+} is distributed between the mobile and stationary phases through the following two equilibria when eluted with a solution of an electrolyte consisting of eluant co-ion Y^{m+} and counter-ion X^{n-}, respectively [1, 2]. Here m^+ and n^- is the charge of eluant ions

$$nC^{p+}(m) + pX^{n-}(m) \rightleftarrows nC^{p+}(s) + pX^{n-}(s) \qquad (1)$$

$$mC^{p+}(m) + pY^{m+}(s) \rightleftarrows mC^{p+}(s) + pY^{m+}(m) \qquad (2)$$

Here, ‘m’ end’s’ represent the mobile phase and the stationary phase, respectively. The first equation indicates the partition equilibria and equation 2 indicates the ion exchange equilibria of a system where Y and X are the eluent cation and anion, and W and Z are another arbitrary eluent cation and anion, and C and A are sample cation and anion respectively. If the size of these ions is large enough so that they cannot penetrate freely into the pores, size exclusion effect must be taken into account. Here, very small size ions are used so that it can be considered size exclusion effect does not contribute to ion retention. The concentration of eluant ion is very higher than the sample ion and assumed that enough to suppress the ion exclusion effect as well. The equilibrium constants of equations (1) and (2) are given by equations (3), (4) and (5) [1, 2, 3]. Here, YX represents the eluant electrolyte.

$$K_{YX} = \left(\frac{\overline{a_Y}}{a_Y}\right)^n \left(\frac{a_X}{a_X}\right)^m \qquad (3)$$

$$K_{CX} = \left(\frac{\overline{a_C}}{a_C}\right)^n \left(\frac{a_X}{a_X}\right)^P \qquad (4)$$

$$K_{C/Y} = \left(\frac{\overline{a_Y}}{a_Y}\right)^n \left(\frac{a_Y}{a_Y}\right)^p \qquad (5)$$

Here, a_i is the activity of ion i, and those with a superscripted bar indicate values in stationary phase. Equations (6), (7) and (8) are not independent from each other, and one of the above three equilibrium constants can be expressed by using the remaining two equilibrium constants

$$K_{YX} = K_{CX}^{\frac{m}{p}} K_{C/Y}^{\frac{-n}{p}} \tag{6}$$

$$K_{CX} = K_{C/Y}^{\frac{n}{m}} K_{YX}^{\frac{p}{n}} \tag{7}$$

$$K_{C/Y} = K_{CX}^{\frac{m}{n}} K_{YX}^{\frac{-p}{n}} \tag{8}$$

In most cases, the concentration of the analyte ion can be neglected compared with that of co-ion in both the mobile and the stationary phase.

$$mc_Y^{YX} = nc_X^{YX} \tag{9}$$

$$m\bar{c}_Y^{YX} = n\bar{c}_X^{YX} \tag{10}$$

where, c_i is the molar concentration of the species i, and the superscript YX represents the eluent electrolyte. From equations (3), (9) and (10), we obtain

$$K_{\mathrm{YX}} = \left(\frac{\bar{a}_X^{YX}}{a_X^{YX}}\right)^{m+n} \left(\frac{\bar{Y}_Y^{YX}\, Y_X^{YX}}{Y_Y^{YX}\, \bar{Y}_X^{YX}}\right)^{n} \tag{11}$$

Where Y_i is the activity coefficient of species i on molarity scale

$$D_C^{YX} = \frac{\bar{c}_C^{YX}}{c_C^{YX}} = \left(\frac{\bar{a}_C^{YX}}{a_C^{YX}}\right)\left(\frac{Y_C^{YX}}{\bar{Y}_C^{YX}}\right) \tag{12}$$

By combining equation (5), (11) and (12)

$$\ln D_{\mathrm{C}}^{\mathrm{YX}} = \ln D_{\mathrm{C}}^{\mathrm{WZ}} + \frac{p}{m+n}(\ln D_{\mathrm{X}}^{\mathrm{WZ}} - \ln D_{\mathrm{Y}}^{\mathrm{WZ}}) \tag{13}$$

where WZ denotes a background eluent electrolyte arbitrarily chosen and $D_{\mathrm{C}}^{\mathrm{WZ}}$, $D_{\mathrm{X}}^{\mathrm{WZ}}$, and $D_{\mathrm{Y}}^{\mathrm{WZ}}$ are the distribution coefficients of C^{p+}, X^{n-}, and Y^{m+} in WZ eluent electrolyte system, respectively. Similarly, the distribution coefficient of an anion A^{p-} when eluted with an electrolyte YX, is expressed as

$$\ln D_{\mathrm{A}}^{\mathrm{YX}} = \ln D_{\mathrm{A}}^{\mathrm{WZ}} + \frac{p}{m+n}(\ln D_{\mathrm{Y}}^{\mathrm{WZ}} - \ln D_{\mathrm{X}}^{\mathrm{WZ}}) \tag{14}$$

Equations (3) and (4) indicate that the distribution coefficient of the analyte ion becomes larger when the mobile phase contains strongly retained counter-ion and weakly retained co-ion and vice versa.

Theoretical equations that describe the retention dependence of ionic solutes on the composition of background electrolytes in the mobile phase have also been developed [3]. In a chromatographic system containing background univalent anions, $\mathrm{X}_1^-\ldots\ \mathrm{X}_N^-$, and a common cation, Y^+, the distribution coefficient of C^{p+} and A^{p-} are given by the following equations, respectively.

$$\ln D_{\mathrm{C}}^{\mathrm{Y}(\mathrm{X}_1+\cdots+\mathrm{X}_N)} = \left(\frac{\sum (D_{\mathrm{C}}^{\mathrm{YX}_i})^{2/p} [\mathrm{X}_i^-]_m}{\sum [\mathrm{X}_i^-]_m} \right)^{p/2} \tag{15}$$

$$\ln D_{\mathrm{A}}^{\mathrm{Y}(\mathrm{X}_1+\cdots+\mathrm{X}_N)} = \left(\frac{\sum [\mathrm{X}_i^-]_m}{\sum \dfrac{[\mathrm{X}_i^-]_m}{(D_{\mathrm{A}}^{\mathrm{YX}_i})^{2/p}}} \right)^{p/2} \tag{16}$$

where $[\mathrm{X}_i^-]_m$ denotes the concentration of anion X_i^- in the mobile phase. Equation (5) shows the dependence of the distribution coefficient of an analyte ion on the composition of the background counter-ions, while equation (6) the dependence on the composition of the co-ions.

From equation (6), it can be obtained the following equation representing the distribution coefficient of the ion A^- in the sample solution zone just after an injection of a large volume of the mixed electrolyte solution of YA and YX into the column [4].

$$\ln D_{\mathrm{A}}^{\mathrm{Y(A+X)}} = \left(\frac{[\mathrm{A}^-]_{smp} + [\mathrm{X}^-]_{smp}}{\dfrac{[\mathrm{A}^-]_{smp}}{(D_{\mathrm{A}}^{\mathrm{YA}})^2} + \dfrac{[\mathrm{X}^-]_{smp}}{(D_{\mathrm{A}}^{\mathrm{YX}})^2}} \right)^{1/2} \tag{17}$$

where $[\mathrm{A}^-]_{smp}$ and $[\mathrm{X}^-]_{smp}$ are the concentrations of A^- and X^- in the sample solution, respectively. When A^- is more strongly retained in the stationary phase than X^-, A^- will migrate slower than X^- in the zone of the mixed electrolytes since $D_{\mathrm{A}}^{\mathrm{YA}} > D_{\mathrm{X}}^{\mathrm{YA}}$ and $D_{\mathrm{A}}^{\mathrm{YX}} > D_{\mathrm{X}}^{\mathrm{YX}}$, and will be continuously separated from the zone during elution through the column. The schematic representation of enrichment of ionic solutes at the trailing edge of co-ion zone has been demonstrated in next page.

Once the ion A^- moves out of the zone, it will begin to migrate at the velocity determined by the distribution coefficient of D_A^{YA} because the background electrolyte is only YA. Since D_A^{YA} is smaller than $D_A^{Y(A+X)}$, the YA zone will move faster than the ion A^- in the mixed electrolyte YA + YX zone. The ion A^- is thus stacked at the trailing edge of the mixed electrolyte zone until the separation of the electrolytes has been completed (Figure 2.1).

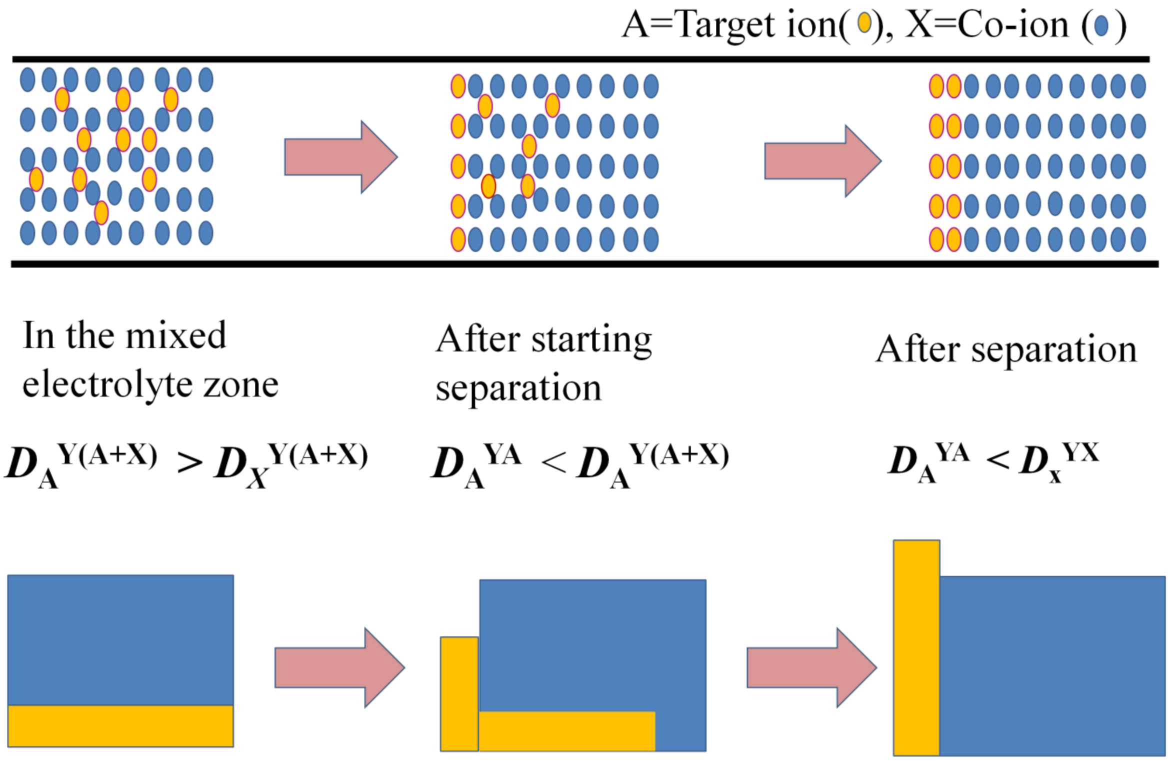

Figure 2.1 Schematic illustration of ion stacking at trailing edge based on partition chromatographic stacking mechanism

In a similar manner, the ion A^- remains should be concentrated at the front edge of the mixed electrolyte zone if it is more weakly retained in the stationary phase than X^-(Figure 2.2). In brief, if $D_A < D_X$, just after injection of the sample solution containing the trace amount of target ion A and co-ion X, then target ion distributes evenly in the solution zone. In the mixed electrolyte zone, the distribution coefficient of analyte ion remains smaller than the distribution coefficient of the co-ion or in other words by the partition effect of co-ion, the migration velocity of target ion becomes larger. When the A has come out of the zone of X, the distribution coefficient of A becomes larger than that exists in the co-ion zone or migration velocity becomes smaller. Thereby, due to the differential migration velocity, A started to stack at the front edge of the mixed electrolyte zone and gradually completely accumulated.

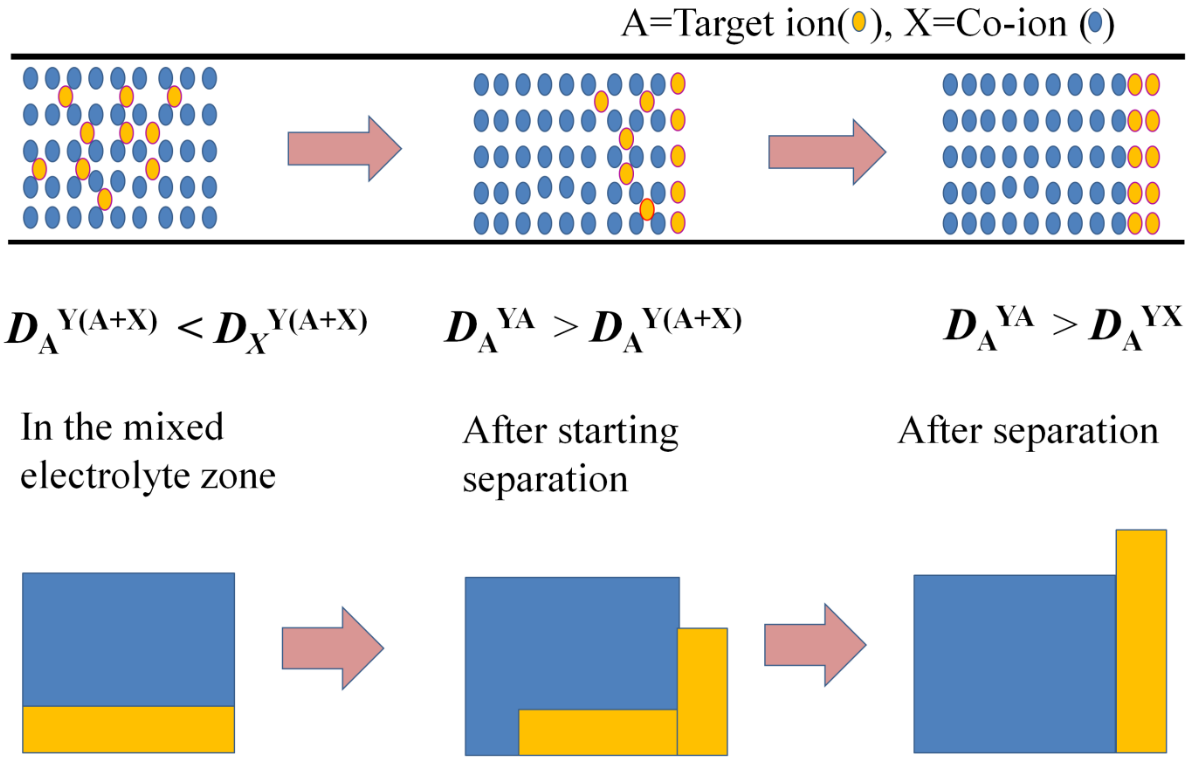

Figure 2.2 Schematic illustration of ion stacking at leading edge based on partition chromatographic stacking mechanism

Figure 2.3 shows a schematic representation of the enrichment behavior of ionic solutes in partition/ion exclusion chromatographic process on a hydrophilic column. As describes earlier, when $D_A^{YA} > D_X^{YX}$, the migration velocity of analyte ion becomes faster after separation from the mixed electrolyte zone and distribution coefficient becomes smaller. In hydrophilic gel as ion exclusion effect is present because of having fixed carboxylic groups on its surface, thus after separation, the A started to migrate not only with the flow of mobile phase velocity but also with an accelerated velocity due to the ion exclusion effect and the distribution coefficient of A becomes much smaller than that when only ion partition model works. Gradually the distribution coefficient of A becomes zero. Therefore, the ion A^- is expected to be stacked in a very narrow zone at the trailing edge of the zone of the mixed electrolyte and high enrichment of A can be attained when ion partition model and ion exclusion effect works in turn.

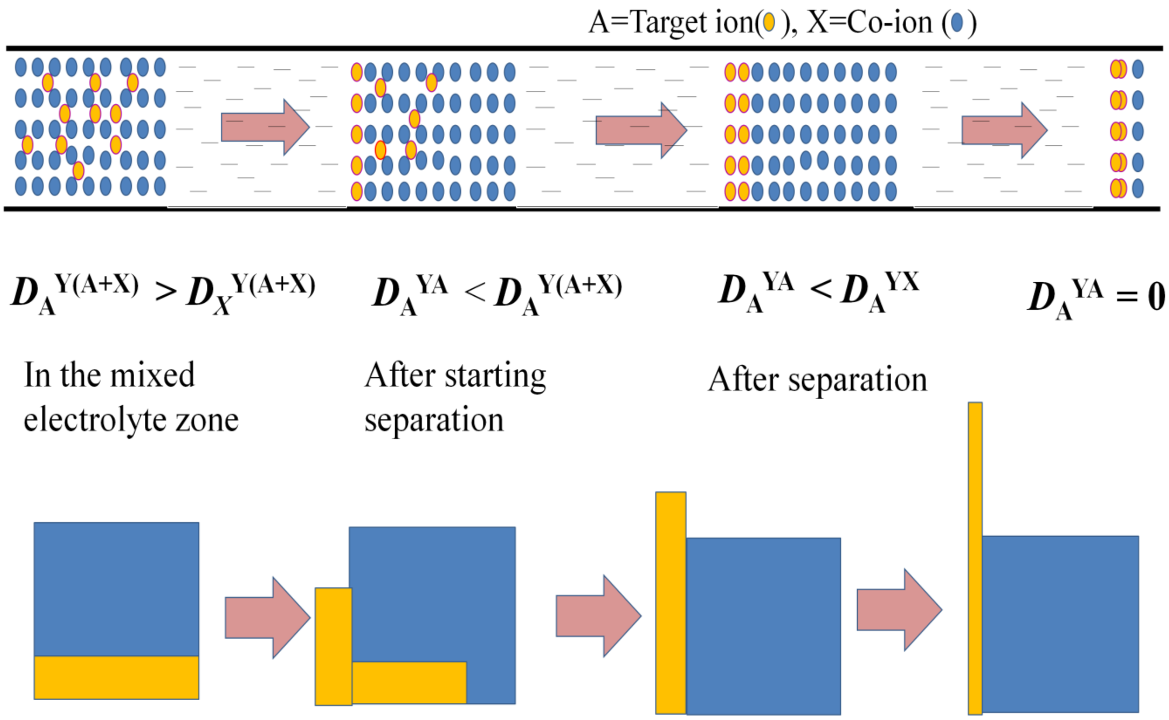

Figure 2.3 Schematic illustration of ion stacking at trailing edge of co-ion based on partition + ion exclusion chromatographic stacking mechanism

2.1 References

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Chapter 3

Ion stacking mechanism on C18 silica and hydroxyl polymethacrylate gels

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3.1 Introduction

From the theory described in Chapters 1 and 2, it is clear that the retention of inorganic ions on the columns packed with the hydrophobic and hydrophilic porous materials is due to the partitioning effect between the bulk liquid phase and the gel phase containing interfacial water. That is, it is expected that the concentration behavior of the electrolyte found by Davankov *et al.* can be observed in any polymer columns regardless of the hydrophilicity and pore size of the packing materials. On the other hand, fixed functional groups exclude the analyte ions of the same charge from the gel phase. However, the added electrolyte to the sample solution suppresses the ion exclusion effect. Hence, when the concentration of analyte is low ion exclusion effect governs the distribution of ion while at higher concentration the partition leads the distribution process of ion.

The former part of this chapter explains the stacking mechanism of anions on a non-ionic hydrophobic C18 bonded silica column based on the partition model using aqueous mobile phase, while in the later part ion stacking method is developed with the ionic hydrophilic gel column employing ion partition model and ion exclusion effects.

3.2 Experimental

3.2.1 Chemicals

All chemicals used in this study were obtained from commercial sources and were of analytical-reagent grade unless otherwise stated. Deuterium oxide (D_2O) was purchased from Wako Pure Chemicals (Tokyo, Japan) for use as a marker of the column void volume or the total water volume in the column. Blue Dextran 2000 obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) was used as a reference material for evaluation of the interstitial volume or interparticulate volume of the column. Water was purified subsequently with an Elix-Advantage

3-UV (Nihon Millipore, Tokyo, Japan) and an Arium 611 DI (Sartorius, Tokyo, Japan) throughout the experiment. The columns used are given in Table 3.1.

Table 3.1 Properties of the columns used

Columns	Material type	Particle size	Column size	Fixed functional group
Capcell Pak C18 UG80	C18 bonded silica	5μm	150 × 4.6mm	
TSKgel SP-STAT	Hydroxylated methacrylic	6 μm	100 × 4.6mm	Sulfonate
Toyopearl HW-40S	Hydroxylated methacrylic	40 μm	100 × 8mm	Carboxyl
TSKgelG2500PW$_{XL}$	Hydroxylated methacrylic	6 μm	300 × 7.8mm	Carboxyl

The chemical structures of the packing materials are given below in Figure 2.1.

Capcell Pak C18 UG80

TSKgel SP-STAT /Toyopearl HW-40S/ TSKgelG2500PW$_{XL}$

Figure 3.1 The Chemical structures of the packing materials used in this study.

3.2.2 Instrumentation and Chromatographic Procedure

The liquid chromatographic system used consisted of a Uniflows (Tokyo, Japan) HPLC pump Model uf-6010 PSW_2, a Hitachi (Tokyo, Japan) L-7420 UV-VIS detector, and a Rheodyne (Cotati, USA) Model 7725i loading injector. All connections between the columns and instruments were made with PEEK tubing. The columns were thermostated at 35 °C using a Shimadzu Model CTO-6A column oven. All the eluents were filtered through a 0.45 µm membrane filter JHWPO 4700 obtained from Nihon Millipore (Yonezawa, Japan) and degassed ultrasonically before use.

Test solutions were prepared by dissolving the salts of analyte ions in water. Each measurement of an analyte ion was conducted at least in triplicate. Data were collected and processed using a CDS plus data analysis system (LA Soft, Tokyo, Japan) or a LC solution system (Shimadzu, Tokyo, Japan).

3.2.3 Determination of cation-exchange capacity of a TSKgel G2500PW_{XL} column

An aqueous solution containing 0.1 mol L^{-1} NaCl with pH adjusted to 9 by the addition of NaOH solution was passed through the column until the pH of the column effluent turned to be 9. After the column was washed with pure water, an HCl solution of pH 3 was passed into the column and the effluent from the column was collected into a 50 mL volumetric flask. The concentration of sodium ion in the solution was determined by means of an inductively coupled plasma atomic emission spectrometer (Seiko Instruments, Tokyo, Japan).

3. 3 Results and Discussion

3.3.1 Stacking behavior of inorganic anions on a C18 silica column

First of all, the ion staking behavior of inorganic anions on a C18 silica column was investigated by introducing a mixed electrolyte solution into the column that had been equilibrated with pure water and then replacing the electrolyte solution with pure water. Figure 3.2 (a) and 3.2 (b) show the elution curves obtained by monitoring the UV absorbance of the column effluent at 235 nm in the process of introduction of a solution containing KIO_3 and $KClO_4$ with a concentration of 1 mmol L^{-1} each to the column and in the replacement or washing process with pure water, respectively. Since perchlorate ion as well as potassium ion does not exhibit UV absorption at this wavelength, these curves represent the variation of the concentration of KIO_3 in the effluent with the elapsed time. It should be noted that the

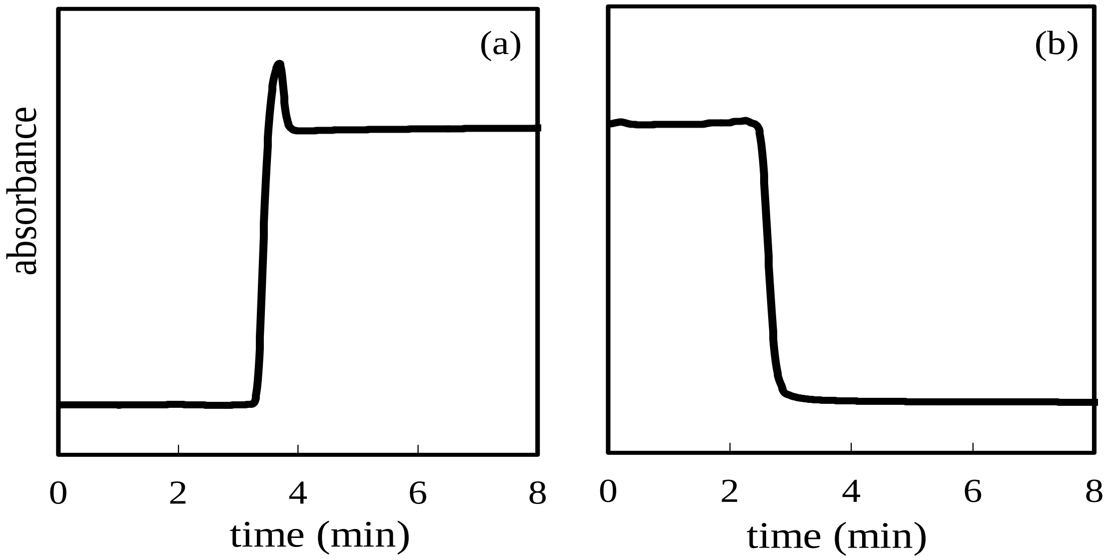

Figure 3.2 Elution curves obtained for the introduction of a solution containing KIO_3 and $KClO_4$ to a C18 silica column equilibrated with water (a) and for the replacement of the solution with pure water (b). Column: Capcell Pak C18 UG80 (5 µm, 150× 4.6 mm). Concentration of the electrolyte in solution: 1 mmol L^{-1}. Flow rate: 0.5 mL min^{-1}. Detection: UV absorption at 235 nm.

concentration of KIO_3 is enhanced at the leading edge of the elution zone (Fig. 3.3(a)), making its concentration larger than the initial concentration in the solution introduced. On the other hand, the trailing edge of the KIO_3 zone shows a regular displacement curve with water (Fig. 3.3(b)). Figure 3.3(a) and 3.3 (b) show the elution curves obtained for the introduction of a solution containing equimolar KCl and KSCN to the column and for the replacement of the solution with pure water, respectively. Figure 3.3 presents the elution behavior of KSCN since only thiocyanate ion exhibits UV absorption at 235 nm in this solution. As can be seen from Fig. 3.3(b), the enhancement of KSCN concentration was observed at the trailing edge of the KSCN zone, contrary to the elution profile of KIO_3 in a mixture with $KClO_4$.

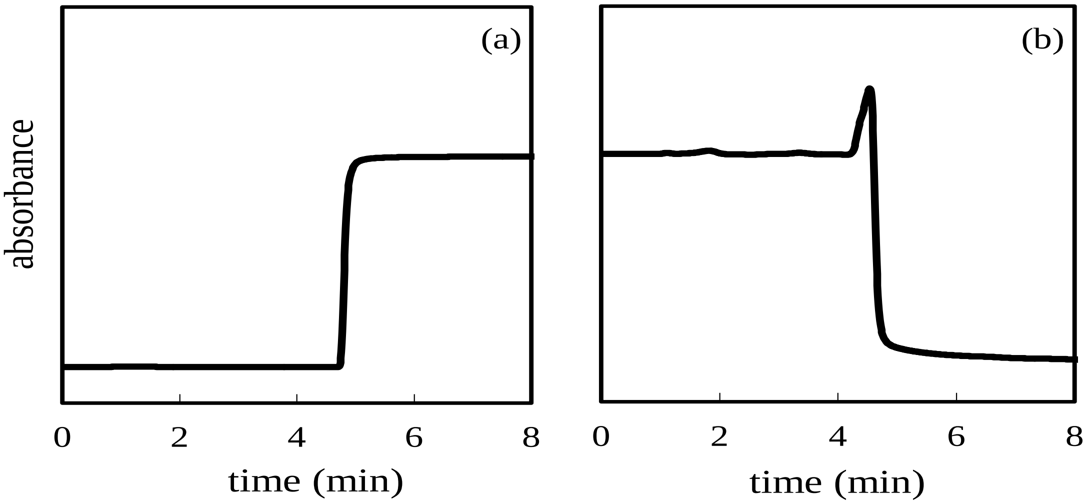

Figure 3.3 Elution curves obtained for the introduction of a solution containing KCl and KSCN to a C18 silica column equilibrated with water (a) and for the replacement of the solution with pure water (b). For other details, see Figure 3.2.

It is impossible to interpret these ion enrichment behaviors with size-exclusion mechanism as stated above. On the other hand, the ion partition model can successfully account for these experimental results. It has been demonstrated that inorganic ions differentiate the interfacial water formed on the surface of C18 silica particles from the bulk water and the chemical separation of the ions in aqueous media with the hydrophobic materials can be interpreted with a partition between the bulk water phase and the interfacial water formed on the hydrophobic surface [1]. The volume of the bulk water in the column can be determined using inorganic ions with the same charge according to the following equation [1, 2, 3]:

$$V_{\mathrm{BW}} = \frac{V_{\mathrm{A}}^{\mathrm{YX}} V_{\mathrm{B}}^{\mathrm{WZ}} - V_{\mathrm{A}}^{\mathrm{WZ}} V_{\mathrm{B}}^{\mathrm{YX}}}{V_{\mathrm{A}}^{\mathrm{YX}} + V_{\mathrm{B}}^{\mathrm{WZ}} - V_{\mathrm{A}}^{\mathrm{WZ}} - V_{\mathrm{B}}^{\mathrm{YX}}} \tag{1}$$

where V_i^{jk} in the right side of the equation is the retention volume of the probe ion *i* obtained by elution with an aqueous solution of the electrolyte *jk*. In this study, univalent inorganic anions, *i.e.*, iodate, bromide, nitrate, iodide, and thiocyanate ions were used as probes and their retention volumes are measured by elution with 0.01 mol L^{-1} NaCl and $NaClO_4$ aqueous solutions for determining the V_{BW} value. The void volume in the column, V_0 was determined, by measuring the retention volume of D_2O and calculated the distribution coefficient of inorganic anions by the following equation [1]:

$$D_{\mathrm{A}}^{\mathrm{YX}} = \frac{C_{\mathrm{A}}^{\mathrm{YX}}(\mathrm{s})}{C_{\mathrm{A}}^{\mathrm{YX}}(\mathrm{m})} = \frac{V_{\mathrm{A}}^{\mathrm{YX}} - V_{\mathrm{BW}}}{V_0 - V_{\mathrm{BW}}} = \frac{V_{\mathrm{A}}^{\mathrm{YX}} - V_{\mathrm{BW}}}{V_{\mathrm{s}}} \tag{2}$$

where $C_{\mathrm{A}}^{\mathrm{YX}}(\mathrm{m})$ and $C_{\mathrm{A}}^{\mathrm{YX}}(\mathrm{s})$ are the concentrations of an anion A^{p-} in the bulk water and the stationary phase water when eluted with an electrolyte YX, respectively, and V_s denotes the volume of the interfacial water that functions as the stationary phase.

In Table 3.2, the distribution coefficients for some inorganic anions determined by elution with aqueous solution of KCl, KIO_3, $KClO_4$ and KSCN are listed. The distribution coefficients of chloride ion and perchlorate ion could not be measured directly in KCl eluant electrolyte system since these anions do not exhibit UV absorption. However, *D* values can be estimated by the following equations because the ratio of the distribution coefficients of two ions with the same charge is constant regardless of the eluent electrolytes [1, 2, 4].

$$D_{\mathrm{Cl}}^{\mathrm{KCl}} = \frac{D_{\mathrm{Cl}}^{\mathrm{KSCN}} D_{\mathrm{IO_3}}^{\mathrm{KCl}}}{D_{\mathrm{IO_3}}^{\mathrm{KSCN}}} \tag{3}$$

$$D_{\mathrm{ClO_4}}^{\mathrm{KCl}} = \frac{D_{\mathrm{ClO_4}}^{\mathrm{KIO_3}} D_{\mathrm{SCN}}^{\mathrm{KCl}}}{D_{\mathrm{SCN}}^{\mathrm{KIO_3}}} \tag{4}$$

Chloride ion in the KSCN eluent system and perchlorate ion in KIO_3 system were detected by indirect UV absorption technique. The $D_{\mathrm{IO_3}}^{\mathrm{KIO_3}}$, $D_{\mathrm{ClO_4}}^{\mathrm{KClO_4}}$, and $D_{\mathrm{SCN}}^{\mathrm{KSCN}}$ were calculated in a similar manner and tabulated in Table 3.2 together with the $D_{\mathrm{ClO_4}}^{\mathrm{KIO_3}}$, $D_{\mathrm{IO_3}}^{\mathrm{KClO_4}}$, and $D_{\mathrm{Cl}}^{\mathrm{KSCN}}$ values as well as $D_{\mathrm{A}}^{\mathrm{KCl}}$. The distribution coefficient apparently increases as the size of the hydrated ion decreases. However, the *D* values of the ions strongly depend on the type of the coexisting counter-ion and co-ion as shown in Table 3.2. These counter-ion and co-ion effects cannot be explained by size-exclusion effect [1, 2, 4, and 5]. The order of the *D* values for ions is not determined by the sizes of the hydrated ions but by the relative solubility of them in the interfacial water at the hydrophobic surface and in the bulk water [1]. The ions having stronger hydration shell show weaker affinity to the interfacial water or smaller distribution coefficients. The effect of the background ions in solution on the distribution of the analyte ion is represented according to equation (3) or (4), which also indicates that the retention of an ionic solute on a column packed with a non-ionic material can rationally be interpreted with the ion partition model [1, 4, 5].

Table 3.2 Distribution coefficients of inorganic anions on a Capcell Pak C18 UG80 with 0.01 mol L^{-1} potassium salt solutions.

Anion	$D_{\mathrm{A}}^{\mathrm{KCl}}$	$D_{\mathrm{A}}^{\mathrm{KIO3}}$	$D_{\mathrm{A}}^{\mathrm{KClO4}}$	$D_{\mathrm{A}}^{\mathrm{KSCN}}$
IO_3^-	0.60	(0.63)[a)]	0.37	
Cl^-	(0.66)[a)]			0.37
Br^-	0.74			
NO_3^-	0.90			
I^-	1.07			
ClO_4^-	(1.57)[a)]	1.72	(1.01)[a)]	
SCN^-	1.99			(1.11)[a)]

a) Calculated values.

From the *D* values shown in Table 3.2, it can be predicted that the distribution coefficient of a component anion in a mixed electrolyte zone is calculated according to equation (5). The calculated distribution coefficients for the component anions in the solution zones of equimolar mixtures of KIO_3 + $KClO_4$ and KCl + KSCN are shown in Table 3.3. The migration velocity, *u*, is related to the distribution coefficient by the following equation:

$$u = \frac{u_0}{1 + D\dfrac{V_{\mathrm{s}}}{V_{\mathrm{BW}}}} \qquad (5)$$

where u_0 is the velocity of the mobile phase. The trailing edge of the KIO_3 zone moves in the solution of the KIO_3 + $KClO_4$ mixed electrolytes, while the leading edge of the zone migrates as pure KIO_3 solution since KIO_3 moves faster than $KClO_4$ and begins to separate just after the introduction of the solution to the column. The distribution coefficients that regulate the migration velocities of the leading edge and the trailing one of the KIO_3 zone is thus given by $D_{\mathrm{IO_3}}^{\mathrm{KIO_3}}$ (= 0.63) and $D_{\mathrm{IO_3}}^{\mathrm{K(IO_3+ClO_4)}}$ (= 0.45), respectively. On the other hand, the leading and trailing edges of the KSCN zone migrate in the column with the velocities determined by the corresponding distribution coefficients, $D_{\mathrm{SCN}}^{\mathrm{K(Cl+SCN)}}$ (= 1.37) and $D_{\mathrm{SCN}}^{\mathrm{KSCN}}$ (= 1.11), respectively, after the introduction of the equimolar mixture of KCl and KSCN. Similarly the zone profiles of

Table 3.3 Calculated distribution coefficients of the component anions in the solution zones of equimolar mixture of KIO_3 + $KClO_4$ and KCl + KSCN on a Capcell Pak C18

Anion	$\boldsymbol{D}_{\mathbf{A}}^{\mathbf{K(IO3+ClO4)}}$	$\boldsymbol{D}_{\mathbf{A}}^{\mathbf{K(Cl+SCN)}}$
IO_3^-	0.45	
ClO_4^-	1.23	
Cl^-		0.45
SCN^-		1.37

$KClO_4$ in the KIO_3 + $KClO_4$ system and of KCl in the KCl + KSCN system can be predicted even though they could not be obtained experimentally; $KClO_4$ should be enriched at the trailing edge of the KIO_3 zone, whereas KCl should be accumulated at the leading edge of the KSCN zone. These results clearly indicate that the ion stacking behavior observed when a mixed electrolyte solution is introduced into the column equilibrated with water can well be explained by the ion partition model; a component electrolyte having smaller distribution coefficient is stacked at the leading edge of the mixed electrolyte zone, while an electrolyte having larger *D* value is accumulated at the trailing edge.

The enhancement of the concentration of a component ion A^- at the leading or trailing edge of the mixed electrolyte (YA + YX) zone depends on the difference in distribution coefficient between the leading edge and the trailing one of the elution zone of YA, that is, the larger the difference between D_A^{YA} and $D_A^{Y(A+X)}$ is, the greater will be the concentration enhancement. The elution curves obtained for the introduction of KIO_3, KNO_3, KI, and KSCN solutions containing equimolar $KClO_4$ to the C18 silica column are shown in Figure 3.4. The order of the anion concentration at the leading edge of the elution zone is IO_3^-> NO_3^- > I^-, although SCN^- is not stacked at the leading edge of its elution zone. This result fits in with the prediction described above.

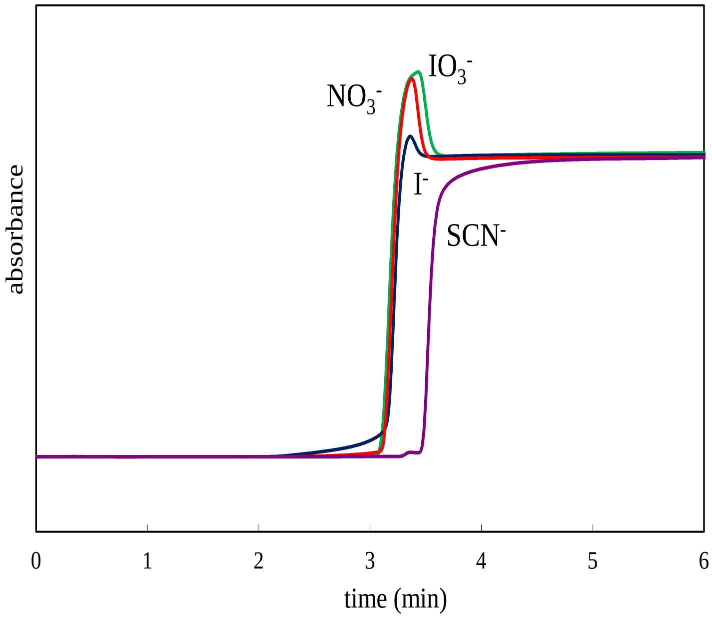

Figure 3.4 Elution curves obtained for the introduction of KIO_3, KNO_3, KI, and KSCN solutions containing equimolar $KClO_4$ to a C18 silica column equilibrated with water. Absorbance values are normalized to the values for the sample solutions introduced. Column: Capcell Pak C18 UG80 (5 μ m, 150 × 4.6 mm). Concentration of the electrolyte in solution: 1 mmol L^{-1}. Flow rate: 0.5 mL min^{-1}. Detection: UV absorption at 235 nm.

3.3.2 Ion stacking by a combination of ion partition and ion exclusion

The results shown above suggest that an effective enrichment of ionic solutes in aqueous solution can be performed only by introducing the solution into a column packed with a nonionic porous material after addition of a suitable electrolyte to the solution and then eluting it with pure water. For ion enrichment columns in practical use, however, packing materials are required that offer high retention capacity as well as sufficient selectivity for different ions. It has been demonstrated that various hydrophilic polymer gels exhibit good retention selectivity for inorganic ions [6-10]. These materials also have high retention capacity because they can absorb a large volume of water, which in turn functions as the stationary phase. The ion enrichment phenomena have so far been observed only on the columns packed with hydrophobic nanoporous materials such as a polystyrene resin [11-13]. It is expected according to the ion partition model, however, that the enrichment of ionic solutes takes place regardless of the hydrophobicity or hydrophilicity of the packing material. The author has thus chosen three cross-linked hydroxylated methacrylic polymer gel column, TSKgel SP-STAT, Toyopearl HW-40S and TSKgel G2500PW$_{XL}$, and attempted to examine the enrichment of inorganic ions. The values of V_0, V_{BW}, V_s, and the interstitial volume (V_{int}) for the TSKgel SP-STAT, Toyopearl HW-40S and TSKgel G2500PW$_{XL}$ columns together with the values for the Capcell Pak C18 UG80 column are given in Table 3.4.

Table 3.4 Void volumes, bulk water volumes, stationary phase water volumes, and the interstitial volumes (mL) for Capcell Pack C18 UG 80, TSKgel SP-STAT, Toyopearl HW-40S, and TSKgel G2500PW$_{XL}$ columns

Columns	**V_o**	**V_{BW}**	**V_S**	**V_{int}**
Capcell Pak C18 UG 80	1.47	1.24 ± 0.03	0.23 ± 0.03	
TSKgel SP-STAT	1.4 ± 0.001	0.511 ± 0.10	0.89	0.471 ± 0.02
Toyopearl HW-40S	3.74 ± 0.03	1.77 ± 0.07	1.97	1.17 ± 0.004
TSK gel G2500PW$_{XL}$	10.22 ± 0.04	6.80 ± 0.18	3.42	5.27 ± 0.05

It has been known that most of organic polymer packing materials contain some fixed anionic charges on their surfaces, usually believed to be carboxyl groups, and may show the ion exclusion effect for anionic solutes at neutral and alkaline conditions [3, 14]. As a result, anions are excluded from the gel phase at low ionic strength, while the partition governs the distribution of anions between the water swollen polymer gel and the bulk solution at high ionic strength. Therefore, if a solution containing a trace amount of YA and a large amount of YX is introduced into a column packed with a polymer gel having fixed anionic groups and eluted with pure water, the anion A^- is expected to migrate at a velocity of u_0 (or larger than u_0) through the column after the separation from the zone of YX. This means that we can achieve higher enrichment of relatively strongly retained anions using the combination of ion partition and ion exclusion by use of a polymer gel column containing a small amount of fixed anionic charge.

Dependence of the retention volumes of NO_3^-, I^-, and SCN^- on the concentration of NaCl in the eluent is shown in Figure 3.5. All the anions show the same retention volumes at NaCl concentration of 1 mmol L^{-1} or less, which are close to the interstitial volume of the columns, for example 5.27 mL for TSKgel G2500 PW_{XL}. The retention volumes of the anions gradually increase with increasing NaCl concentration in the eluent, reaching their respective constant values at the NaCl concentration of 100 mmol L^{-1} or above. This result demonstrates that the anions are completely excluded from the interior of the polymer gel particles by the ion exclusion effect of the fixed ions at the eluent ionic strength of 1 mmol L^{-1} or below, while the effect of the fixed charges is negligible and the anionic solutes can partition into the gel phase at 100 mmol L^{-1} or above. The cation-exchange capacity determined for the columns are given in Table 3.5.

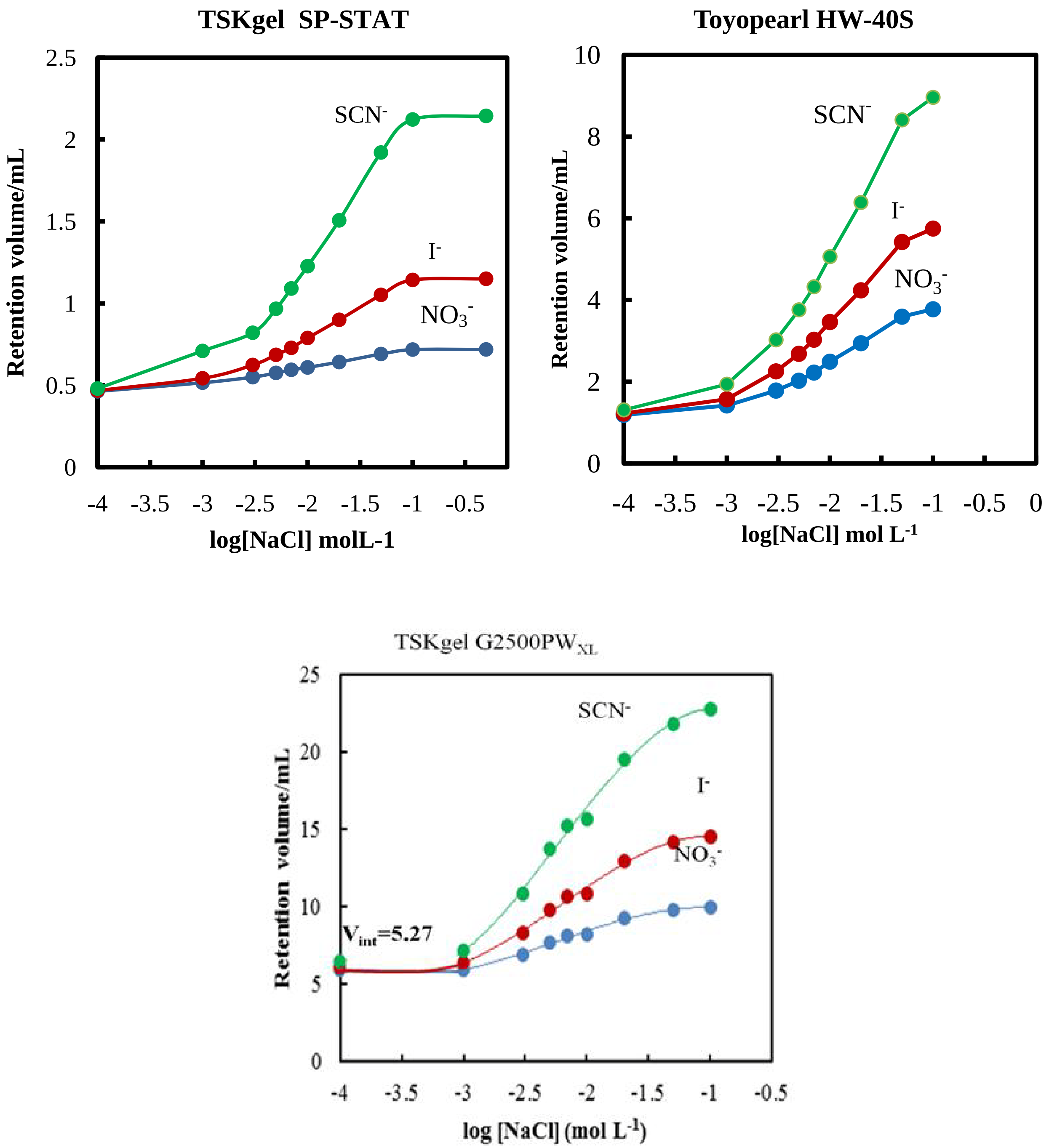

Figure 3.5 Dependence of the retention volumes of NO_3^-, I^-, and SCN^- on the eluent electrolyte concentration. Column: TSKgel SP-STAT, Toyopearl HW-40S and TSKgel G2500PW$_{XL}$. Eluent electrolyte : NaCl.

Table 3.5

Ion exchange capacities determined for TSKgel SP-STAT, Toyopearl HW-40S and TSKgel G2500PW$_{XL}$ columns.

Column	**µeq/mL**
TSKgel SP-STAT	40.7 ± 0.15
Toyopeal HW-40 S	5.2 ± 0.05
TSKgel G2500PW$_{XL}$	3.4 ± 0.03

As seen from the Table, TSKgel SP-STAT column has comparatively larger cation exchange capacity than the other two columns. The distribution coefficients obtained for some inorganic anions by elution with 0.1 mol L^{-1} NaCl and Na_2SO_4 are listed in Table 3.6. By comparison of the distribution coefficients shown in Table 3.6 with those in Table 3.2, it is apparent that the TSKgel G2500PW$_{XL}$ and Toyopearl HW-40S columns have higher retention selectivity for inorganic anions than that of a C18 silica column. As shown in the previous section, the larger the difference between the distribution coefficients of a target ion and a coexisting ion, the enhancement of the concentration of the target ion at the leading or trailing edge of the mixed electrolyte zone is greater. Therefore, higher enrichment of target ions, not only due to the ion exclusion effect but also to the larger co-ion effect, can be expected to be obtained by use of the TSKgel G2500PW$_{XL}$ and Toyopearl HW-40S columns instead of the Capcell Pak C18 UG80 and TSKgel SP-STAT columns.

Table 3.6 Distribution coefficients of anions with 0.1 mol L^{-1} NaCl and Na_2SO_4 solutions

Anions	**TSKgel SP-STAT**		**Toyopearl HW-40S**		**TSKgel G2500PW$_{XL}$**	
	$\mathbf{D_A^{NaCl}}$	$\mathbf{D_A^{Na2SO4}}$	$\mathbf{D_A^{NaCl}}$	$\mathbf{D_A^{Na2SO4}}$	$\boldsymbol{D_A^{NaCl}}$	$\boldsymbol{D_A^{Na2SO4}}$
SO_4^{2-}	(0.03)[a]	(0.03)[a]	(0.12)[a]	(0.14)[a]	(0.11)a)	(0.32)a)
IO_3^-	0.04	0.05	0.30	0.43	0.2	0.42
Cl^-	(0.10)[a]	(0.35)[a]	(0.69)[a]	(0.28)[a]	(0.36)a)	(0.60)a)
Br^-	0.16	0.36	0.81	0.72	0.71	1.42
NO_3^-	0.23	0.54	0.98	0.91	0.93	1.8
I^-	0.71	1.87	1.85	1.91	2.27	4.42
SCN^-	1.81	4.47	3.39	3.66	4.66	8.99

a) Calculated values.

When a target ion A^- is introduced into the column as a solution containing the mixed electrolytes of a trace amount of YA and a large amount of YX, the distribution coefficient of the ion A^- in the zone of the electrolyte YX is expected to be D_A^{YX}. On the other hand, the distribution coefficient of the ion X^- or the electrolyte YX is given by D_X^{YX}. When D_A^{YX} is larger than D_X^{YX}, the ion A^- is expected to be stacked in a very narrow zone at the trailing edge of the zone of the co-existing electrolyte YX, since the ion A^- will move much faster than the trailing edge of the electrolyte zone due to the ion exclusion effect after it has been separated out from YX. Figure 3.6 (A) and 3.6 (B) show the chromatograms of nitrate ion obtained by injecting a solution containing 1 μmol L^{-1} $NaNO_3$ and 0.1 mol L^{-1} NaCl into the TSKgel G2500PW$_{XL}$ and Toyopearl HW-40S columns and then eluting it with pure water, respectively.

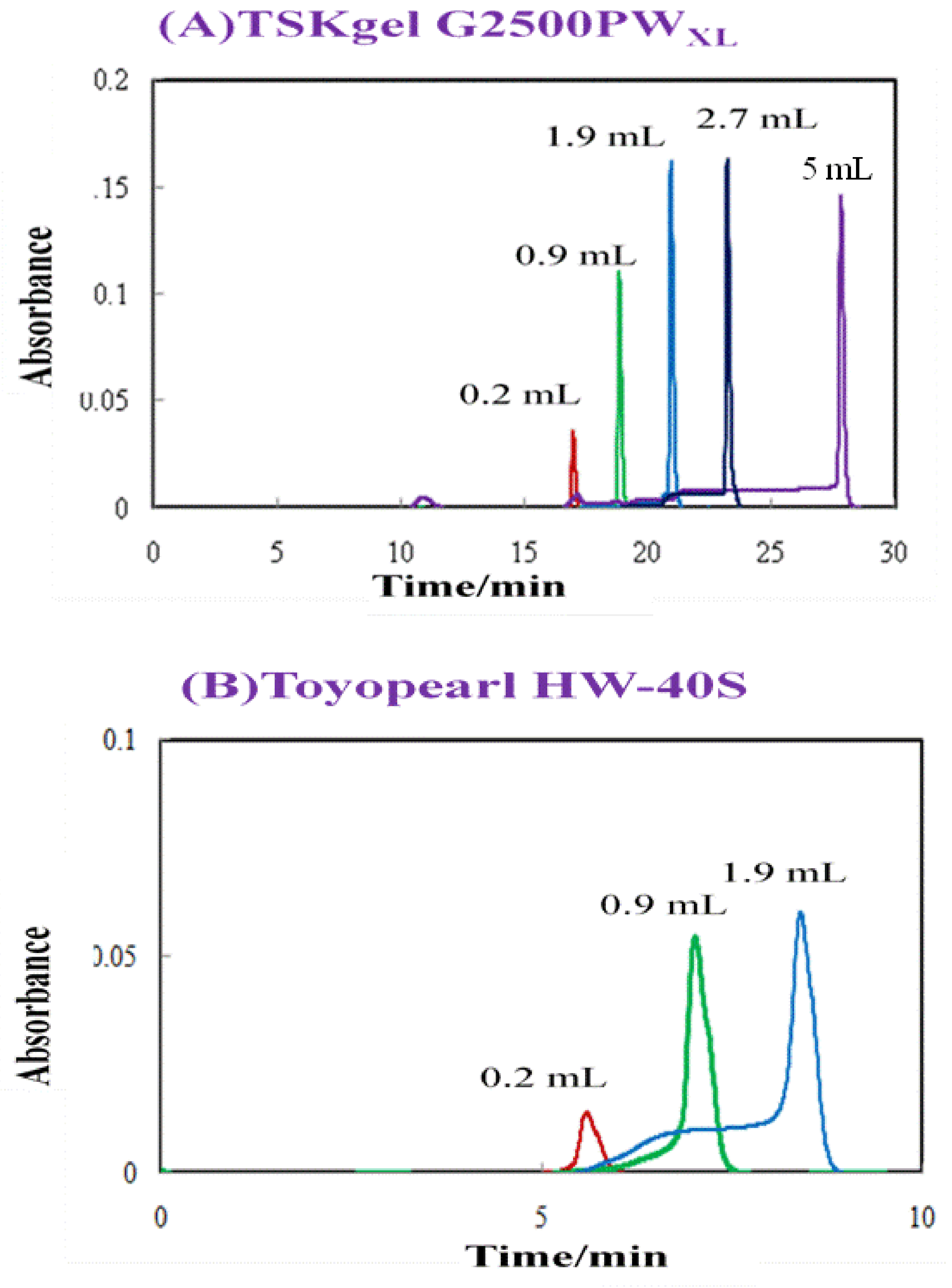

Figure 3.6 Variation of the Chromatograms of NO_3^- obtained by injecting a mixed electrolyte solution of $NaNO_3$ + NaCl with the sample volume injected. Column: TSKgel G2500PW$_{XL}$ (A) and Toyopearl HW-40S (B). Concentration of electrolyte in the sample solution: $NaNO_3$ = 1 μmol L^{-1}; NaCl = 0.1 mol L^{-1}. Flow rate: 0.5 mLmin^{-1}.

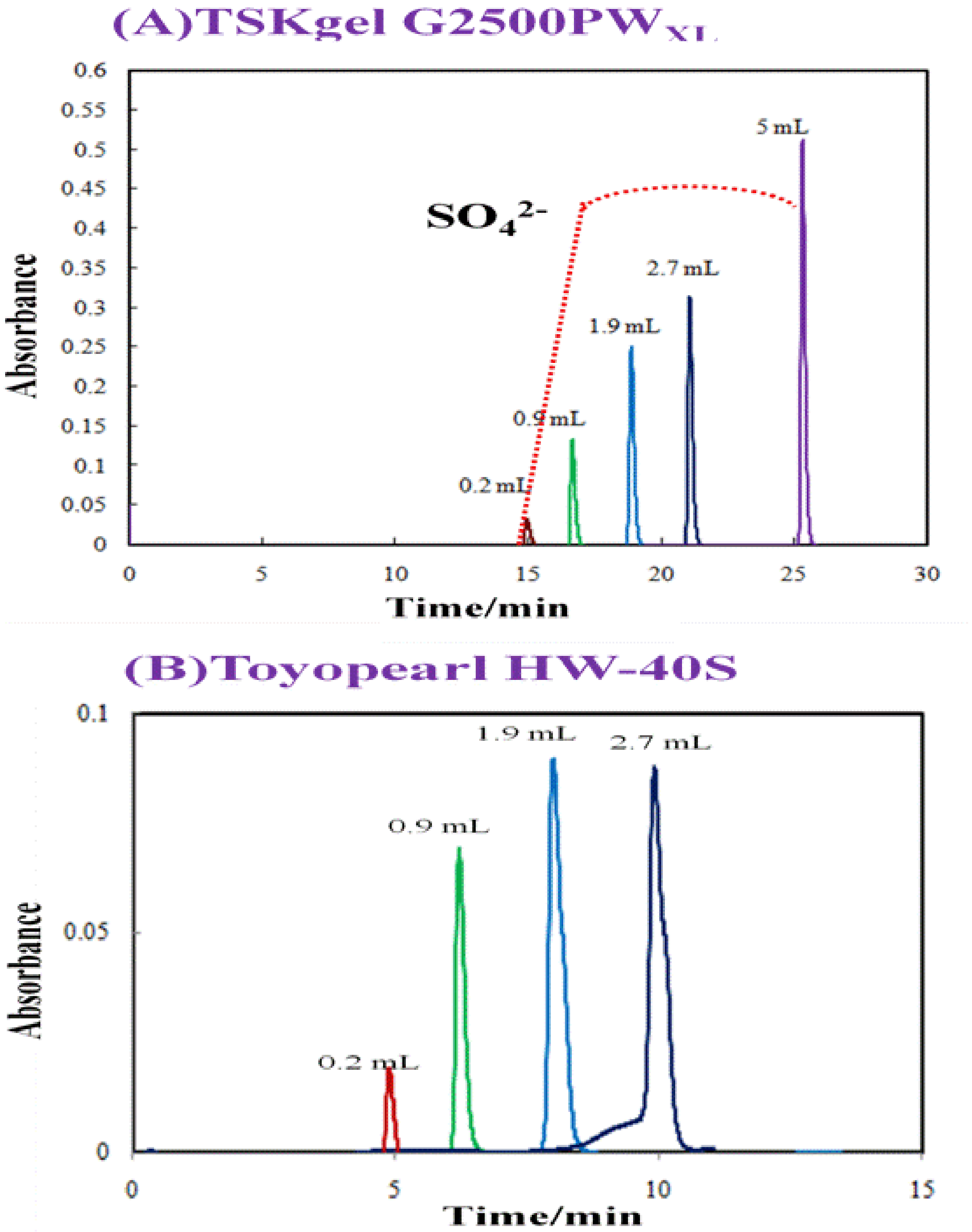

Figure 3.7 **V**ariation of the Chromatogram of NO_3^- obtained by injecting a mixed electrolyte solution of $NaNO_3$ + Na_2SO_4 with the sample volume injected. Column: TSKgel G2500PW_{XL} (A) and Toyopearl HW-40S (B). Concentration of electrolyte in the sample solution: $NaNO_3$= 1 μmol L^{-1}; Na_2SO_4 = 0.1 mol L^{-1}. Flow rate: 0.5 $mLmin^{-1}$.

Sharp peaks for nitrate ion were detected regardless of the sample volume and the peak height increased with an increase in the sample volume injected up to 0.9 ml and 1.9 mL for Toyoperal HW-40S and TSKgel G2500PW$_{\mathrm{XL}}$, respectively.

On the other hand, the peak obtained by injecting the solution with 2.7 or 5.0 mL onto TSKgel G2500PW$_{\mathrm{XL}}$ was accompanied by a broadened leading zone, which started to elute at the retention time for the peak obtained by injecting 1.9 mL of the solution. For Toyoperal HW-40S, the broadened peak appears when the sample solution with more than 0.9 mL is injected. This indicates that nitrate ion is accumulated at the trailing edge of the zone of NaCl by the partition effect and stacked in the narrow zone by the ion exclusion effect. Without the ion exclusion effect, the width of the nitrate zone would increase with an increase in the volume of the solution injected, because of pure NaNO$_3$ zone, which would start to be formed just after the injection of the solution, migrate slower than the trailing edge of NaCl zone, *i.e.*, $D_{\mathrm{NO_3}}^{\mathrm{NaNO_3}} > D_{\mathrm{Cl}}^{\mathrm{NaCl}}$.

The rather small difference in the distribution coefficient between NO$_3^-$ and Cl$^-$ in the NaCl zone, *i.e.*, $D_{\mathrm{NO_3}}^{\mathrm{NaCl}}$ and $D_{\mathrm{Cl}}^{\mathrm{NaCl}}$, accounts for incomplete stacking of nitrate ion for an injection of the solution more than 0.9 mL on Toyoperal HW-40S or 1.9 mL on TSKgel G2500PW$_{\mathrm{XL}}$. When $D_{\mathrm{A}}^{\mathrm{YX}} > D_{\mathrm{X}}^{\mathrm{YX}}$, the retention volume of the leading edge of the zone of a target ion A$^-$ obtained by an injection of the solution containing YA and an excess amount of YX can be assumed to be approximately equal to $V_{\mathrm{A}}^{\mathrm{YX}}$ given by

$$V_{\mathrm{A}}^{\mathrm{YX}} = V_{\mathrm{BW}} + D_{\mathrm{A}}^{\mathrm{YX}} V_{\mathrm{s}} \qquad (6)$$

On the other hand, the retention volume of the trailing edge of the zone of YX, $V_{\mathrm{tr(YX)}}$ is represented by

$$V_{\mathrm{tr(YX)}} = V_{\mathrm{BW}} + D_{\mathrm{X}}^{\mathrm{YX}} V_{\mathrm{s}} + V_{smp} = V_{\mathrm{X}}^{\mathrm{YX}} + V_{smp} \qquad (7)$$

where V_{smp} is the volume of the sample solution injected. Equations (6) and (7) indicate that the overall stacking of the target ion injected can be performed when $V_{smp} \leq V_{\mathrm{A}}^{\mathrm{YX}} - V_{\mathrm{X}}^{\mathrm{YX}}$. If the sample volume is so large that $V_{\mathrm{tr(YX)}}$ exceeds $V_{\mathrm{A}}^{\mathrm{YX}}$, the complete enrichment of the target ion into a single narrow zone will not be possible. The $V_{\mathrm{NO_3}}^{\mathrm{NaCl}}$ and $V_{\mathrm{Cl}}^{\mathrm{NaCl}}$ values on Toyopearl HW-40S are 3.71 mL and 2.77 mL, while the $V_{\mathrm{NO_3}}^{\mathrm{NaCl}}$ and $V_{\mathrm{Cl}}^{\mathrm{NaCl}}$ values on TSKgel G2500PW$_{\mathrm{XL}}$ are

9.98 and 8.03 mL, respectively. Therefore, the upper limit of the sample volume for the complete stacking of nitrate ion is thus calculated to be 0.94 mL on Toyopearl HW-40S, and 1.95 mL on TSKgel G2500PW$_{XL}$ for the solution added with NaCl. This prediction agrees well with the experimental results shown in Figure 3.6 (A) and 3.6 (B). The variation of the chromatograms of nitrate ion with the sample volume injected for a solution containing 1 μmol L^{-1} $NaNO_3$ and 0.1mol L^{-1} Na_2SO_4 is shown in Figure 3.7. Nitrate ion showed a very sharp peak even when 1.9 mL and 5.0 mL of the sample solution were injected into the Toyoperal HW-40S and TSKgel G2500PW$_{XL}$ columns. These results are ascribed to the fact that $D_{NO_3}^{Na_2SO_4}$ is much larger than $D_{SO_4}^{Na_2SO_4}$ (Table 3.6) and indicate that one should add an anion of which the distribution coefficient is much smaller than that of the target anion to the sample solution for higher enrichment of the target anion.

The enrichment factor, *E* was calculated as the ratio of the absorbance at the top of the peak of a target ion and the absorbance shown by the initial sample solution. Figure 3.8 shows the relationship of the enrichment factor of nitrate with the volume of the sample solution with

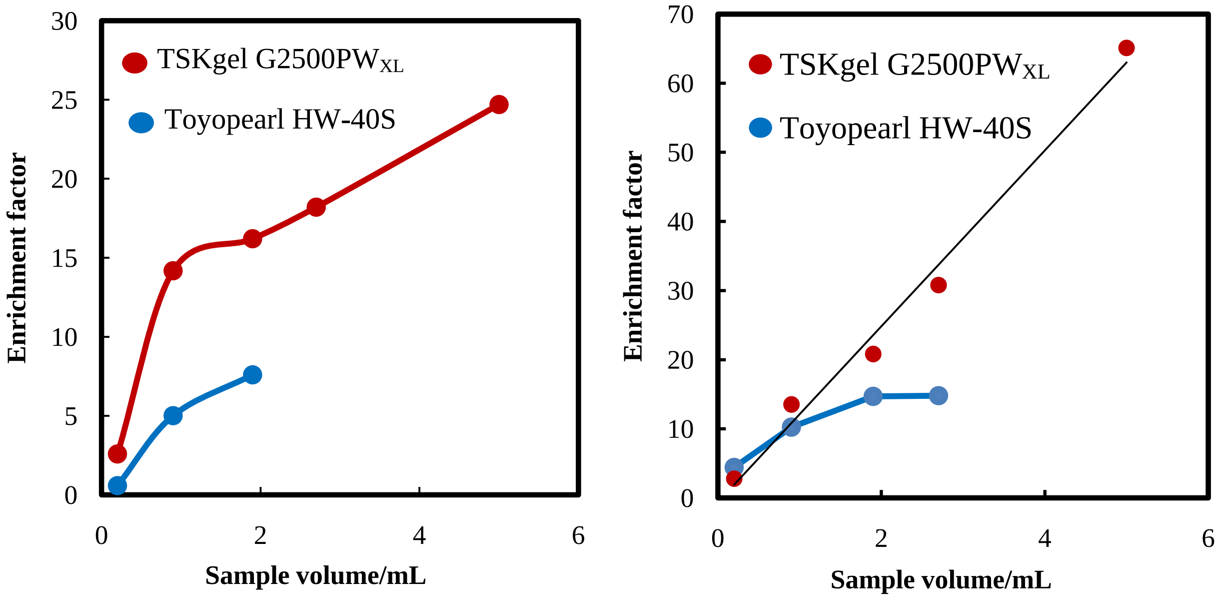

Figure 3.8 Relationships between the *E* and the sample volume injected for **NO_3^- with added NaCl (A) and Na_2SO_4 (B)**. Columns: TSKgel G2500PW$_{XL}$, Toyopearl HW-40S. Mobile phase: pure water. Concentration of the target ion in the sample solution = 1 μmol L^{-1}. Concentration of the added background electrolyte in the sample solution = 0.1 mol L^{-1}

added Na_2SO_4 and NaCl on TSKgel G2500PW$_{XL}$ and Toyopearl HW-40S, while Figure 3.9 and 3.10 show the relationship between the enrichment factor and sample volume for iodide and thiocyanate. As can be seen from Figure 3.8, all the plots, except for the NaCl-added nitrate ion solution, give straight lines of approximately the same slope of *ca*. 12 mL^{-1} and *ca.* 6 mL^{-1} on TSKgel G2500PW$_{XL}$ and Toyopearl HW-40S, respectively. This means that the width of the stacked ion zone does not depend on the sample volume injected but is kept constant without dispersion during migration in the column.

On the other hand, as shown in Figures 3.9 and 3.10, all the plots attain straight lines not only for TSKgel G2500PW$_{XL}$ but also for Toyopearl HW-40S when Iodide and thiocyanate solutions were injected. This is attributed to the larger distribution coefficients of iodide and thiocyanate than chlorite and sulphate as shown in Table 3.6.

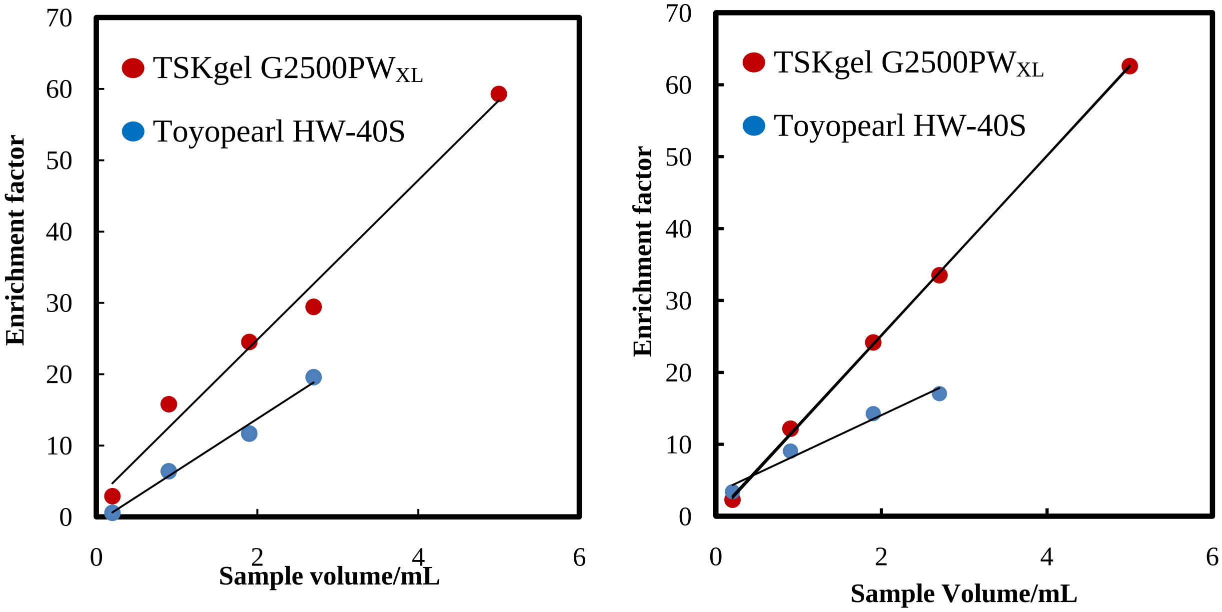

Figure 3.9 Relationships between the enrichment factor and the sample volume injected for **I^- with added NaCl (A) and Na_2SO_4 (B)**. Columns: TSKgel G2500PW$_{XL}$, Toyopearl HW-40S. Mobile phase: pure water. Concentration of the target ion in the sample solution = 1 µmol L^{-1}. Concentration of the added background electrolyte in the sample solution = 0.1 mol L^{-1}

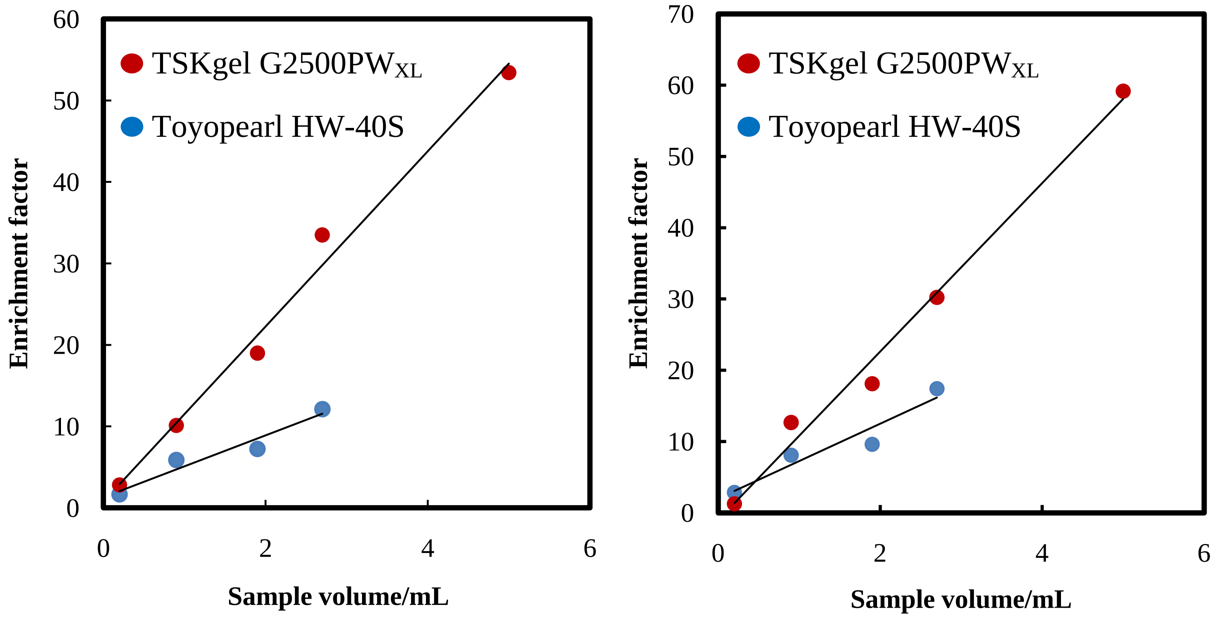

Figure 3.10 Relationships between the enrichment factor and the sample volume injected for **SCN$^-$ with added NaCl (A) and Na$_2$SO$_4$ (B)**. Columns : TSKgel G2500PW$_{XL}$, Toyopearl HW-40S. Mobile phase: pure water. Concentration of the target ion in the sample solution = 1 µmol L^{-1}. Concentration of the added background electrolyte in the sample solution = 0.1 mol L^{-1}

We may thus easily achieve over a hundred-fold enrichment of ionic solutes using this simple ion stacking method provided that the ionic strength of the accumulated target ion zone remains within the range where the ion exclusion effect is predominant (1 mmol L^{-1} or less for the TSKgel G2500PW$_{XL}$ column). The higher enrichment factor can be obtained with the added Na$_2$SO$_4$ solution since the difference in distribution coefficient between the target ion (A = NO$_3^-$, I$^-$, and SCN$^-$) and the co-ion (SO$_4^{2-}$) is large. The estimated enrichment factor that can be attained is given in Table 3.7.

Table 3.7 Estimated maximum enrichment factors for NO_3^-, I^- and SCN^- for Toyopearl HW-40S and TSKgel G2500PW$_{XL}$ columns

Anions	Toyopearl HW-40S		TSKgel G2500PW$_{XL}$	
	Sample volume/mL	*E*	Sample volume/mL	*E*
NO_3^-	1.5	6.2	5.0	60
I^-	3.4	15.3	14.0	170
SCN^-	6.9	30.5	29.7	350

Table 3.7 shows that the maximum enrichment factor obtained with the TSKgel G2500PW$_{XL}$ column is larger than that with Toyopearl HW-40S. This is because the column efficiency of TSKgel G2500PW$_{XL}$ is larger or the particle size is smaller than that of Toyopearl HW-40S. By the use of larger column packed with smaller particles one can obtain the larger enrichment factor by this simple enrichment method.

3.4 Conclusions

In the present study, the author have demonstrated that the stacking of component ions in an aqueous mixed electrolyte solution observed during elution in the chromatographic column packed with a non-ionic material can successfully be interpreted with the ion partition model [1, 4]. The ion stacking takes place by the change in the distribution of the ions between the bulk water and the water incorporated in the packing material, which acts as the stationary phase, by the effect of the co-ions in the solution. When an aqueous solution containing ions to which an excess amount of an electrolyte has been added is injected into the column and eluted with pure water, the ions exhibiting weaker retention than the added co-ion are stacked at the leading edge of the added electrolyte zone, while the other ions showing stronger retention are accumulated at the trailing edge.

The author has developed a useful method for enrichment of trace amounts of anions using the combination of the chromatographic ion stacking and ion exclusion effect of fixed anionic charges on a hydrophilic polymer packing material. The anions in a sample solution having the distribution coefficients larger than that of the anion of the added electrolyte are concentrated in a very narrow zone at the trailing edge of the elution band of the electrolyte. The enrichment factor as high as several hundred for some inorganic anions could be achieved with a hydrophilic polymer column of 300 mm long. The use of a larger column may enable one to obtain much higher enrichment factor. The ion enrichment method developed in this study is very simple, cost effective, and versatile since only pure water and a simple inorganic salt suitable for the enrichment of a target ion are needed and the operation can be performed in a single run, which discloses the possibility for preparatory scale enrichment.

3.5 References

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Chapter 4

Determination of trace amounts of ions in water and salt samples using partition / ion exclusion chromatographic ion stacking

4.1 Introduction

The author has succeeded in accumulating analyte anions having the distribution coefficients larger than that of the anion of the added electrolyte in a very narrow zone at the trailing edge of the elution band of the electrolyte using ion-exclusion effect exerted by fixed anionic charges on a hydrophilic polymer packing material as well as the partition chromatographic ion stacking [1]. It was shown that the enrichment factor as high as several hundred for some inorganic anions could be achieved with a hydrophilic polymer column of 300 mm long. This ion enrichment method is very simple, cost effective, and versatile since only pure water and a simple inorganic salt suitable for the enrichment of the analyte ions are needed and the operation can be performed in a single run.

It is thus expected that this partition/ion-exclusion chromatographic ion stacking method (PIEC stacking) can be used for preconcentration of trace amounts of analyte anions in water samples. The PIEC stacking may also be used for the analysis of some trace impurity anions in salt samples if the impurity anions are retained more strongly than the component anion of the samples salt on the hydrophilic polymer column. The impurity anions will be accumulated at the trailing edge of the salt solution band only by injecting an aqueous solution of the salt into the column and eluting it with pure water.

In this chapter, the author presents a new analytical methodology using the PIEC ion stacking for simple and efficient on-line preconcentration of trace inorganic anions in water and salt samples prior to ion chromatographic determination. The developed on-line PIEC ion stacking–ion chromatography (IC) method was successfully applied to the determination of nitrate ion in tap water and impurity nitrite and nitrate in reagent grade sodium salts, NaCl and Na_2SO_4.

4.2 Experimental

4.2.1 Chemicals

Water was purified with an Elix-Advantage 3-UV (Nihon Millipore, Tokyo, Japan) and an Arium 611 DI (Sartorius, Tokyo, Japan) throughout the experiment. All chemicals were of JIS guaranteed reagent (GR) grade and extra pure (EP) grade, and were purchased from Wako (Osaka, Japan) or Kanto Chemicals (Tokyo, Japan) unless otherwise stated. Suprapur (SP) grade anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany).

4.2.2 Instrumentation and Chromatographic Procedure

The setup for on-line PIEC ion stacking-IC was made up of two liquid chromatography systems and a motorized 2-piston 10-port valve connected as shown in Figure 4.1. The first dimension HPLC system for preconcentration of analyte anions consisted of a Uniflows (Tokyo,

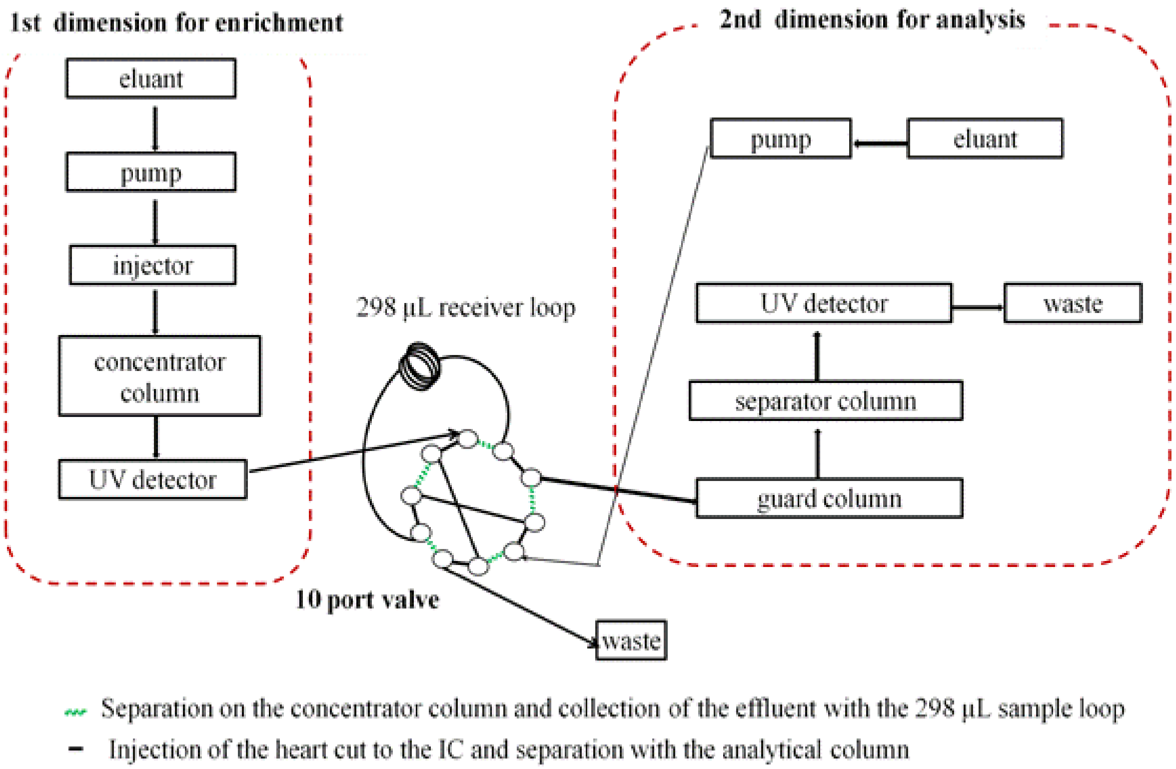
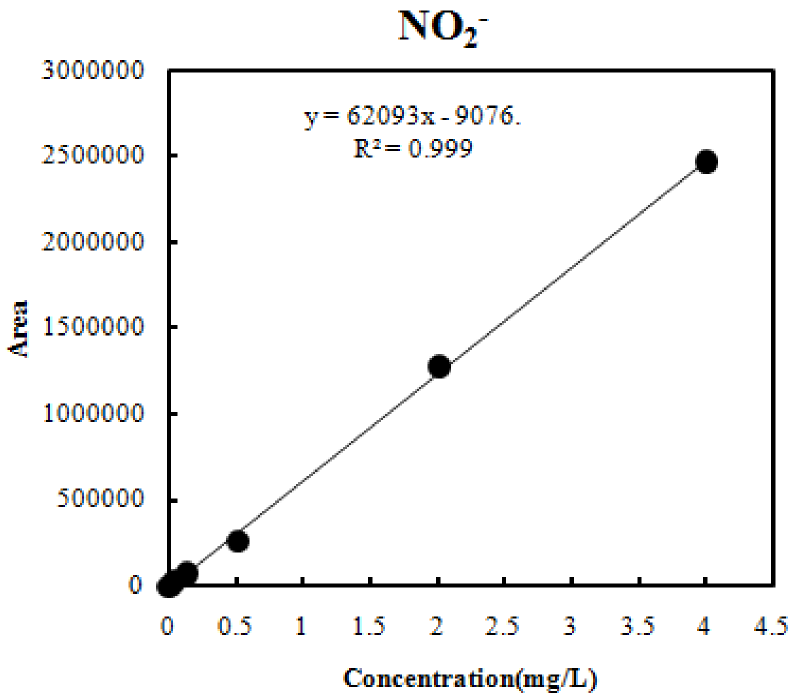


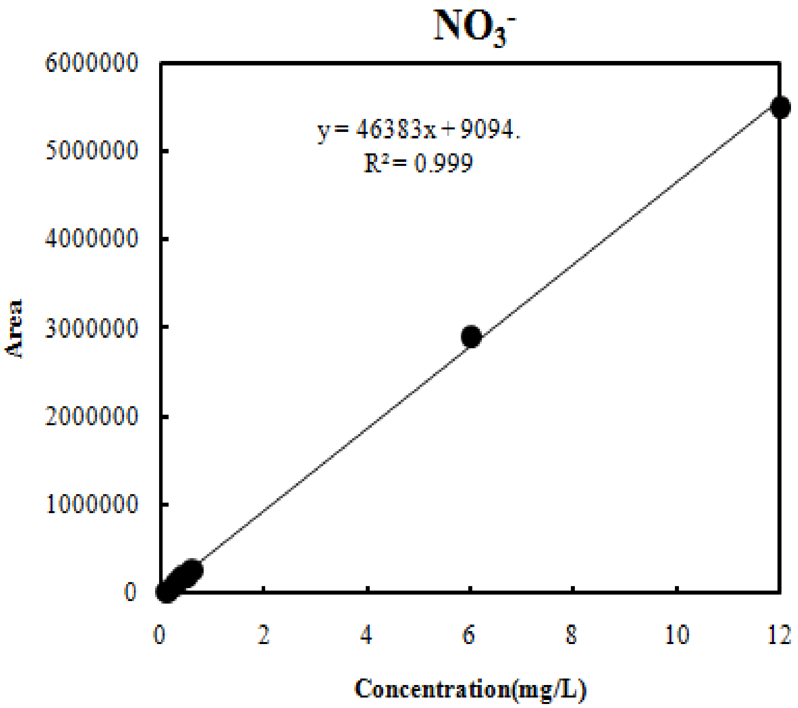
Figure 4.1 Instrumental set up of on-line PIEC ion stacking–IC.

Japan) HPLC pump Model uf-6010 PSW_2, a Senshu Scientific (Tokyo, Japan) Model 3000B UV detector, and a Rheodyne (Cotati, USA) Model 7725i loading injector. The column used as the anion concentrator was a TSKgel G2500PW_{XL} (cross-linked hydroxylated methacylate polymer gel, 7.8 × 300 mm) was obtained from Tosoh (Tokyo, Japan). The second dimension IC system used for determination of trace anions was a Dionex Model ICS-1600 (California, USA) consisting of a dual-piston pump, a column heater, an AS 22 guard column (4 × 50 mm) and an AS 22 separator column (4 × 250 mm). A Hitachi (Tokyo, Japan) model L-7420 UV-VIS detector was used for detection of the analyte anions. All connections between the columns and instruments were made with polyether ether ketone (PEEK) or Teflon tubing. Pure water and 4.5 mM Na_2CO_3 - 1.4 mM $NaHCO_3$ were used as the eluants for anion enrichment (the first dimension) and the IC determination (the second dimension), respectively. The fraction of the effluent from the concentrator column was collected in a 298 µL loop attached to the 10 port valve and then transported to the IC column. The time of switching valve was optimized in such a way that the fraction of the effluent containing the entire concentrated zone was collected into the receiver loop. Each measurement of an analyte ion was conducted at least in triplicate. Data were collected and processed using a CDS plus data analysis system (LA Soft, Tokyo, Japan).

4.2.3 Preparation of samples and working standard solutions

Tap water was collected from the laboratory and filtered through a 0.45 µm membrane filter to remove suspended particulate matter. A 1 mL aliquot of the filtrate tap water sample was diluted with pure water or 0.100 M aqueous solution of SP grade Na_2SO_4 to 50 mL and kept in polyethylene bottles in cool place (refrigerator). The tap water solution diluted with pure water was used for the direct determination of nitrate ion by IC, while the solution prepared with 0.100 M Na_2SO_4 was for the analysis by on-line PIEC ion stacking-IC. The sample solutions of reagent grade Na_2SO_4 and NaCl were prepared by dissolving the salts with pure water to give the concentration of 0.100 M. Working solutions were prepared by appropriate dilution of the standard solution (1000 mg/L) for nitrite and nitrate obtained from Wako Pure Chemicals (Tokyo, Japan) with pure water. For recovery measurements, samples were spiked with nitrite or nitrate by adding diluted standard solutions at two different concentration levels. The calibration curves that were used to determine of the concentration of inorganic anions (NO_2^-, NO_3^-) using standard solutions are shown below.





4.3 Results and Discussion

4.3.1 Determination of nitrate ion in tap water

According to the explanation given in chapter 3, the width of the stacked zone does not depend on the volume of the sample solution injected, which is *ca.* 170 μL for the TSKgel G2500PWXL column used in the present study. Therefore it is desirable that the component anion of the background electrolyte has as small a distribution coefficient as possible in order to achieve higher enrichment of the target anions by PIEC stacking. In the previous study [1] , the author demonstrated that sulfate ion exhibits the smallest distribution coefficient (= 0.32) among common inorganic anions and the overall stacking can be achieved for anions having the larger than 1.78 such as nitrate, iodide, thiocyanate with the TSKgel G2500PW$_{XL}$ column (V_s = 3.42 mL) even when 5.00 mL of sample solution was injected. Thus Na_2SO_4 was adopted as the background electrolyte to be added to the sample solution and validated the on-line PIEC ion stacking-IC method for the accuracy and reproducibility by determining nitrate ion in tap water.

Figure 4.2 shows the chromatogram recorded on the first concentrator column by elution with pure water for an injection of 5.00 mL of tap water diluted 50-fold with 0.100 M Na_2SO_4. Thus, the author used SP grade Na_2SO_4 in order to minimize the contamination of nitrate from the added salt. A sharp single peak observed on the chromatogram can be attributed to trace anions in the tap water sample

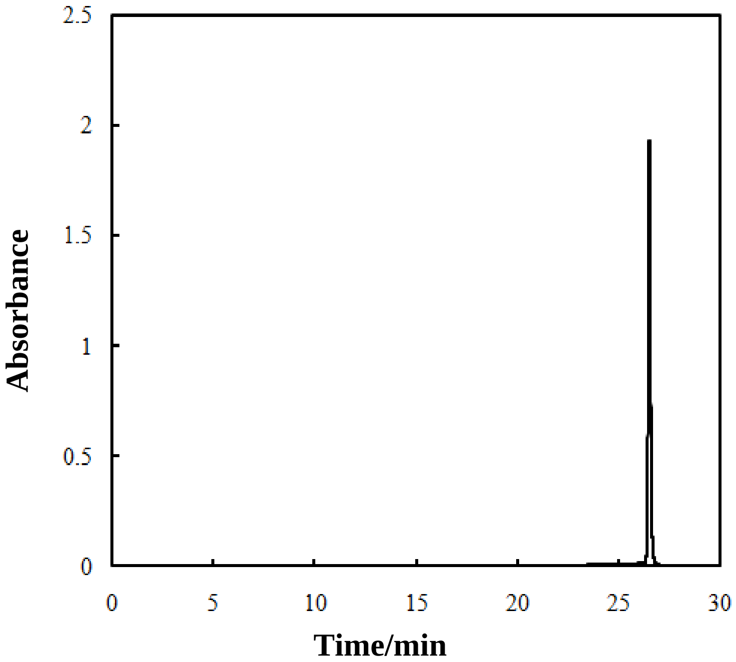


Figure 4.2 Chromatogram obtained for an injection of 50-fold diluted tap water with 0.100 M Na_2SO_4 into the concentrator column. Injection volume: 5 mL, eluent: pure water, flow rate: 0.5 mL min^{-1}, detection wavelength: 220 nm.

accumulated at the trailing edge of the elution band of Na_2SO_4, of which the width at half height is *ca.* 10 min or 5 mL. The entire stacked zone containing trace anions was heart-cut by means of a 10-port valve mounted with a 298 μL loop and transferred to the IC for analysis.

Shown in Figure 4.3 is the chromatogram of the tap water sample obtained by on-line PIEC ion stacking-IC. The peak for nitrate ion was observed in front of a negative peak caused by elution of sulfate that coeluted with nitrate on the concentrator column. The chromatogram obtained by IC for an injection of 298 μL of the tap water diluted with pure water by a factor of 50 is shown in Figure 4.4 for comparison. As can be seen, the peak area for nitrate obtained by on-line PIEC ion stacking-IC is significantly larger than that by IC owing to the preconcentration by PIEC ion stacking; the enrichment factor is given to be 16.8 by the volume ratio of the sample solution injected (5.00 mL) to the heart-cut volume (298 μL).

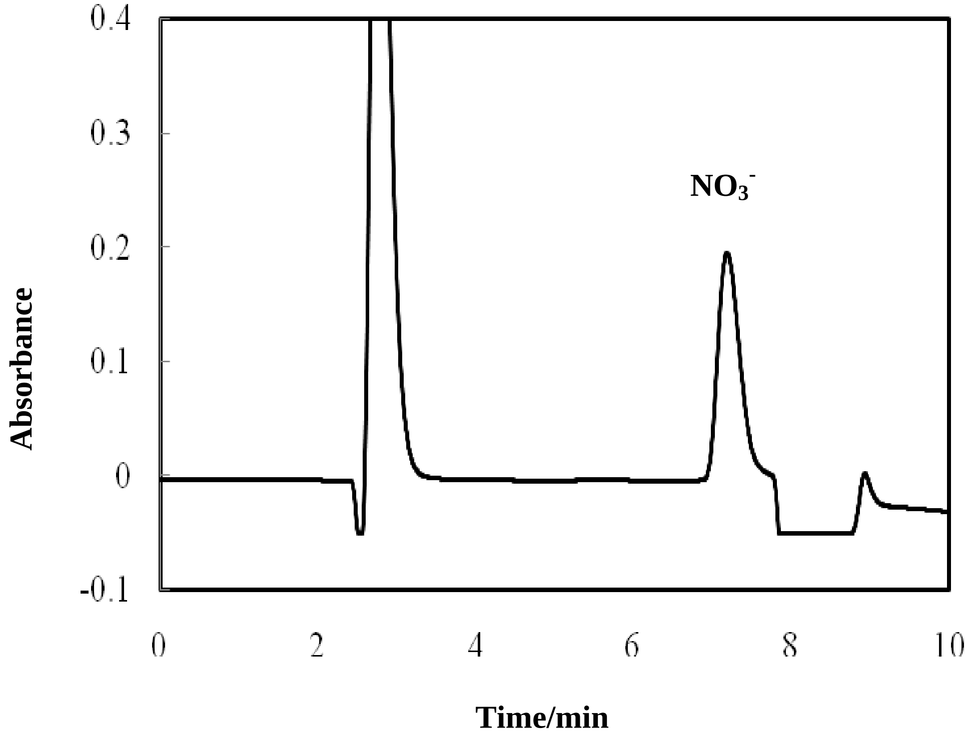


Figure 4.3 Chromatogram of 50-fold diluted tap water with 0.100 M Na_2SO_4 obtained by on-line PIEC ion stacking-IC. Eluent: 4.5 mM Na_2CO_3 + 1.4 mM $NaHCO_3$, flow rate: 1.2 mL min^{-1}, detection wavelength: 220 nm.

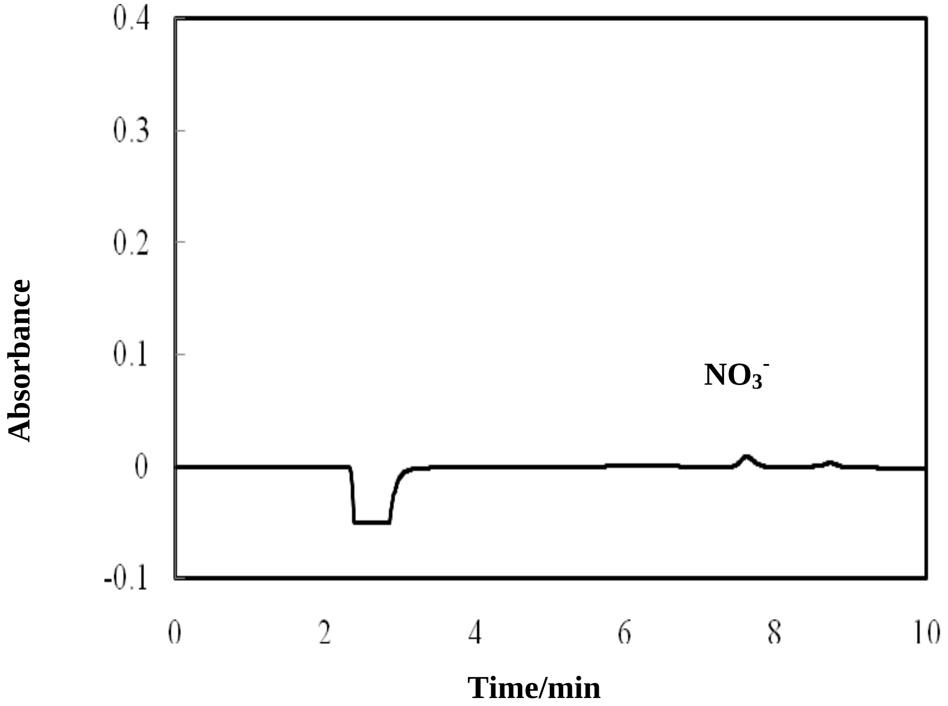


Figure 4.4 Chromatogram of 50-fold diluted tap water with water obtained by direct injection into the separator column. Injection volume: 298 µL, eluent: 4.5 mM Na_2CO_3 + 1.4 mM $NaHCO_3$, flow rate: 1.2 mL min^{-1}, detection wavelength: 220 nm.

Table 4.1 Results of the recovery test for nitrate determination (*n* = 3) in a 50-fold diluted tap water

Sample	Ion	Added (mgL^{-1})	Found (mgL^{-1})	Recovery (%)
Tap water	NO_3^-	0	0.122 ± 0.001	-
		0.050	0.173 ± 0.002	105 ± 2
		0.100	0.219 ± 0.001	97 ± 2

Spike recovery tests were performed to evaluate the accuracy of the method. The results of the spike recovery for injections of 5.00 mL tap water sample diluted with 0.1 M Na_2SO_4 are given in Table 4.1. The values of recoveries of nitrate are satisfactory and low standard deviation values of measurements indicate good precision of the developed on-line PIEC ion stacking-IC method. The detection limit was determined by injecting 0.100 M solution of SP grade Na_2SO_4 salt into the on-line PIEC ion stacking-IC system. The detection limit defined as three-fold of the standard deviation of the blank signal ($n = 3$) was 0.5 µg L^{-1}. These results indicate that the on-line PIEC ion stacking–IC method is useful for enrichment and determination of trace anions in water samples.

4. 3. 2 Determination of trace anions in reagent grade salts

It is expected that on-line PIEC ion stacking–IC can also be used for determination of trace impurity anions in salt samples because one can analyze the trace anionic contents of matrices which contain high concentrations of component ions by direct injection of the salt solutions into the on-line PIEC ion stacking–IC system. The author has thus attempted to determine trace anions in GR and EP grade salts of Na_2SO_4 and NaCl by on-line PIEC ion stacking–IC. The maximum injection volume of NaCl solution for overall stacking of nitrate is calculated to be 1.95 mL from the distribution coefficients of chloride and nitrate ions, $D_{\mathrm{Cl}}^{\mathrm{NaCl}}$ (= 0.36) and $D_{\mathrm{NO_3}}^{\mathrm{NaCl}}$ (= 0.93). Consequently, the author injected 1.90 mL of the NaCl solution into the on-line PIEC ion stacking–IC system for the analysis of trace anions in the reagent grade salts of NaCl.

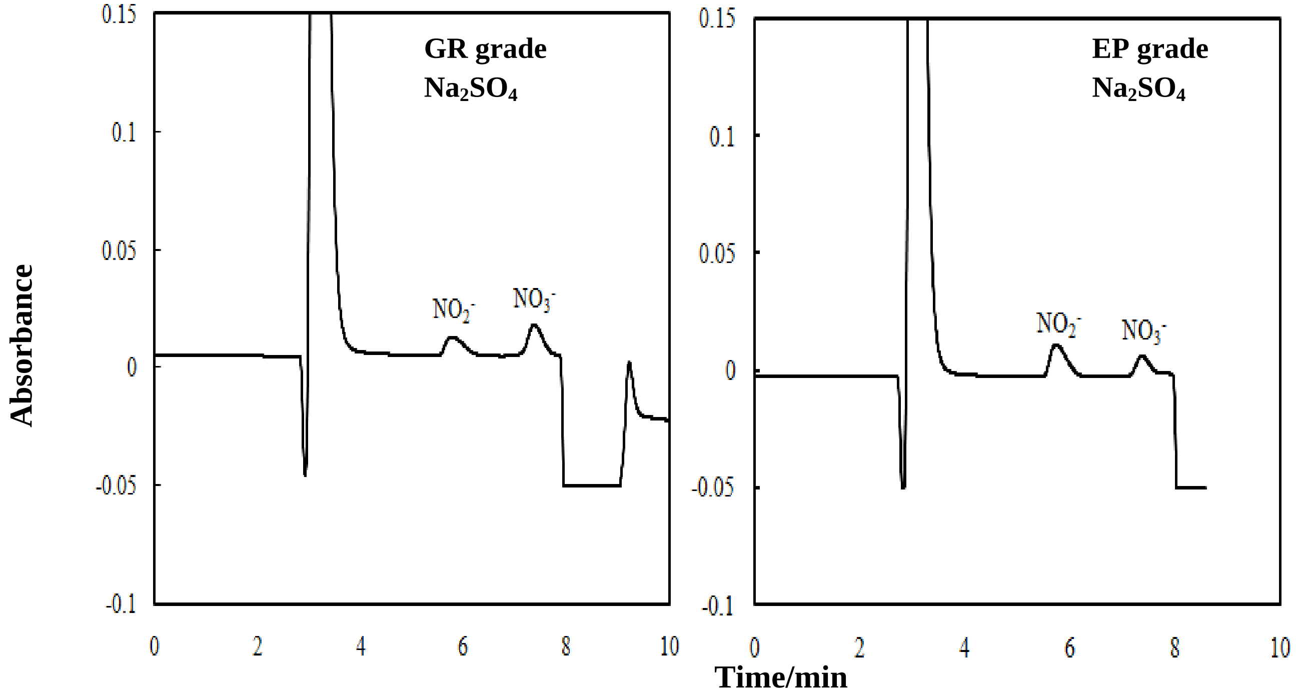


Figure 4.5 Chromatograms obtained for injections of 0.100M GR and EP grade Na_2SO_4 solutions by on-line PIEC ion stacking-IC. For other details see Figure 3.

Figure 4.5 shows the chromatograms obtained for injections of 0.100M aqueous solutions of the two different grade salts of Na_2SO_4. The peaks for nitrite and nitrate were detected for both GR and EP grade salts of Na_2SO_4, while no distinct peaks were observed on the chromatograms of the NaCl solutions partly because the enrichment factor is not sufficiently high. The results of spike recovery tests are summarized in Table 4.2. The distribution coefficient of nitrite ($D_{NO_2}^{Na_2SO_4}$ = 1.62) in Na_2SO_4 background electrolyte system is a little smaller than that of nitrate and the V_{smp} value calculated according to the equation of previous chapter, for nitrite it is 4.45 mL. Therefore, the complete accumulation of nitrite into the stacked zone was not achieved. However, the mass fraction of nitrite collected in the receiver-loop of 298 µL is calculated to be 95 % (= (4.45 + 0.30)/5.00). As can be seen, the recoveries were 94-100% for all the measurements although the values for nitrate determination are around 95 % as predicted.

Table 4.2 Results of the recovery test for nitrate and nitrite determination (*n*=3) in 0.100 M NaCl and Na_2SO_4 solutions

			GR grade		EP Grade	
Salts	Ions	Added (µg/L)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)
Na_2SO_4	NO_3^-	0	10.3 ± 0.1	-	6.7 ± 0.2	-
		10	20.5 ± 0.1	102 ± 1	17.2 ± 0.5	105 ± 4
		20	31.4 ± 0.3	105 ± 1	26.1 ± 0.4	97 ± 2
	NO_2^-	0	6.9 ± 0.1	-	13.0 ± 0.5	-
		5	11.6 ± 0.4	94 ± 8	17.8 ± 0.6	96 ± 12
		10	16.5 ± 0.3	96 ± 3	26.1 ± 0.4	97 ± 3
NaCl	NO_3^-	0	n.d.[a]	-	n.d.[a]	-
		10	9.7 ± 0.2	97 ± 2	9.6 ± 0.5	96 ± 5
		20	21.2 ± 0.3	106 ± 1	19.9 ± 0.2	100 ± 1

[a] not detected

Since pure water is used as the eluent for the PIEC ion stacking, extremely low background level can be achieved by the present method. The detection limits for nitrite and nitrate in the analysis of reagent grade Na_2SO_4 were 0.02μg L^{-1} and 0.04μg L^{-1}, respectively. The contents of impurity nitrite and nitrate in the GR and EP grade salts of Na_2SO_4 were calculated as the mass percentage of $NaNO_2$ and $NaNO_3$ in the sodium salt samples (Table 4.3) assuming that the recovery for both the anions is 100 %. These impurities were found to be within the limits specified by the manufacturers (N-nitrogenous compounds in Na_2SO_4 : 5 ppm; N-nitrogenous compounds in NaCl : 1 ppm [2]). The results shown in Tables 4.2 and 4.3 reveal that trace impurity anions present at sub-ppm level in salt samples can be determined by on-line PIEC ion stacking–IC.

Table 4.3 Quantification results of GR and EP grade sodium sulfate for impurity $NaNO_2$ and $NaNO_3$.

Salt	GR grade(mg/kg)	EP Grade(mg/kg)
$NaNO_3$	1.01 ± 0.01	0.66 ± 0.01
$NaNO_2$	0.73 ± 0.01	1.39 ± 0.05

4.4 Conclusions

A novel method, on-line PIEC ion stacking–IC, for simple and sensitive determination of trace inorganic anions in water and salt samples has been proposed. This method is based on ion chromatography with an on-line preconcentration of the target anions by partition/ion-exclusion chromatographic ion stacking. Target trace anions are concentrated on a hydrophilic polymer gel column containing a small amount of fixed anionic charges during elution with water in the presence of large amount of background electrolyte. Subsequently the stacked anion zone is transferred to the ion chromatography column for separation and determination. The on-line PIEC ion stacking-IC method developed in this work has been validated by testing its recovery, precision, and detection limit values for determination of nitrate in a tap water sample.

This method has also been demonstrated effective for determination of impurity nitrate and nitrite present at sub-ppm level in the reagent grade salts of Na_2SO_4 and NaCl. The salt samples used in this study may contain other impurity anions than nitrite and nitrate. However, they could not be detected because some of them may not be concentrated at the trailing edge of the background electrolyte band due to their small distribution coefficients on the concentrator column and the others may coelute with the background co-ion, *i.e.*, Cl^- or SO_4^{2-} on the separator column. Ongoing work is underway to more fully explore new capability of the developed method to broaden the range of applications.

4. 5 References

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2. http://www.e-reagent.com/uh/Zkk.do?now=1517384711997

Chapter 5

Conclusions and Outlook

The author has developed an ion stacking method for enrichment of ionic solutes in aqueous solution with a water incorporated hydrophilic polymer gel column utilizing partition of ions between bulk water and gel phase consisting of polymer matrix and sorbed water as well as ion exclusion effect exerted by the fixed anionic groups of the polymer column. It has been demonstrated that the stacking of ionic solutes can successfully be accounted by the ion partition model in terms of distribution coefficient and the elution time of the target ion can be predicted by the model as well. The effective concentration of anionic solutes in aqueous solutions was performed only by introducing the solution into a hydrophilic polymer column after addition of a suitable electrolyte to the solution and then eluting it with pure water. High enrichment factor of inorganic anions was obtained only by injection of large amount of mixed sample solution into a larger polymer column packed with smaller particles. Based on the results, it is possible to say that enrichment of ions depends on the injection volume for the particular column. In addition, this ion enrichment effect is not limited to non-ionic C18 silica and ionic hydroxylated methacylic polymer material but should be observed for any materials if water is incorporated / delivered to the column regardless of hydrophilicity and pore size of the packing material.

The developed aqueous partition chromatographic ion stacking–ion chromatography method was also validated by recovery experiments for determination of nitrate in tap water in terms of accuracy and precision and the results showed the reliability of the method. The method proposed was also successfully applied to the determination of trace impurity nitrite and nitrate in reagent grade salts of sodium sulfate. This method provides a new tool to the sensitive determination of impurity anions in GR and EP grade salts as well. This method is expected to be implemented for purification of electrolytes if the fraction of effluent of concentrator column containing the leading pure zone is collected manually and injected several times into the PIEC-ion chromatographic system.

Since pure water is used as the eluent in the concentrator column, extremely low background level can be achieved by the developed method. But from the results it can be said that high background level of Na_2SO_4 in separator column interferes with the detection of target anions even though they are enriched in the concentrator column. In order to have low background conductivity in the separator column, salts of weak acids should be added as the background electrolyte to the sample solution before injection into the concentrator column. Successful detection of more trace anions may broaden the range of application.

Ion partition chromatographic stacking mechanism is one of the on-column concentration methods, which can minimize the loss of the analytes in the preconcentration process. However, very few, if any, effective and versatile methods for on-column concentration of trace anions have been reported to the best of my knowledge. In addition, the principle of this method is also expected to be applied to the enrichment of cations using anion exchanger hydrophilic gel column and eluting with pure water.

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The Author

List of Publications

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1. Mechanism of ion stacking in aqueous partition chromatographic processes, Fouzia Akter, Yawara Ogiyama, Shingo Saito, Masami Shibukawa, *Journal of Separation Science*, **40(16),** 3205-3213 (2017).

2. Partition/ion-exclusion chromatographic ion stacking for analysis of trace anions in water and salt samples by ion chromatography, Fouzia Akter, Shingo Saito, Yuiko Tasaki-Handa, Masami Shibukawa, *Analytical Science*, **34(3),** 369-373 (2018).

National Conferences

1) F. Akter, S. Saito, M. Shibukawa, The 76th Analytical Chemistry Discussion, D 2006 (p.69), May, 2016, Gifu University, Gifu, Japan (Oral).

2) F. Akter, S. Saito, M. Shibukawa, Eastern Japan Analysis Young Professional Exchange Association, P16 (p.40), July, 2016, Nihon University, Karuizawa Training Center, Karuizawa, Japan (Poster).

3) F. Akter, S. Saito, Y. Handa, M. Shibukawa, Eastern Japan Analysis Young Professional Exchange Association, July, 2017, Shiobara, Japan (Poster).

4**)** F. Akter, S. Saito, Y. Handa, M. Shibukawa, The 66th Annual Meeting of the Japan Society for Analytical Chemistry, September, 2017, Tokyo University of Science, Katsushika Campus, Tokyo, Japan **(**Poster).

International Conferences

1) F. Akter, S. Saito, M. Shibukawa, the 13th Asian Conference on Analytical Sciences, OR11 (p.101), December 2016, Chiang Mai, Thailand (Oral).