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学位論文題目	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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論文の内容の要旨

Enhancing the detection sensitivity of trace amount of ions in a sample containing large amounts of co-existing components is a common problem prior to analysis. Although a lot of spectrophotometric, electrochemical, solid-phase extraction (SPE), liquid-liquid extraction methods are used for enrichment of inorganic and organic ions in aqueous samples, ion chromatography (IC) and integrated online SPE-IC have gained immense attention for various applications and are often regarded as most acceptable techniques for monitoring trace amounts of inorganic ions in water samples. Despite the well performance of the aforementioned methods, some bottlenecks such as low sensitivity, use of organic solvents and/or toxic reagents for extraction or elution of the analyte ions, sample loss, *etc.* make them expensive, tedious, and time consuming. 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.

A unique on-column ion stacking phenomenon in the liquid chromatographic process on non-ionic nanoporous material has been reported. According to the method, enhancement of the concentrations of the component ions in the sample solution containing two different electrolytes takes place when the solution is introduced into a column packed with a hydrophobic non-ionic polystyrene material and eluted with pure water. The separation and enrichment behaviors of inorganic ions exerted by the porous material were explained in terms of size-exclusion mechanism based on hydration radii of ions. In brief, this explanation is based on the fact that ions with larger hydrated radii usually show smaller retention volumes. But it has been reported in many papers that the separation of ionic solutes not only with hydrophobic polystyrene materials but also with silica and other hydrophilic porous materials such as cross-linked dextran, polyacrylamide gels, *etc.* cannot be interpreted by size-exclusion mechanism. An enrichment of solute compounds of large molecular size may take place if a dry porous material is used and large molecules are excluded from the pores. But in the procedure reported, analyte ions were injected into a water incorporated column and elution was carried out with

pure water, that is, the pores of the materials had already been filled with water before injection of the ions. In addition, it was assumed that the polystyrene resin functions as an inert material and the state of water in the pores was the same as that of the bulk water. Usually, hydrogen bonds present in water reform 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.

On the other hand, Shibukawa, *et al.* showed that this structure perturbed water at hydrophobic and hydrophilic surfaces has different affinity to ionic solutes than the bulk water and the ion can partition between the bulk water and the interfacial water. They also estimated the amount of water which exhibits different affinity to ionic solutes by a liquid chromatographic technique. Furthermore, they succeeded in interpreting the chemical separation of ionic solutes with a stoichiometric ion partition model.

According to their ion partition model, the retention of an ionic solute on a polymer gel column depends on the nature of counter-ion and co-ion in the mobile phase and these background ion effects are interpreted with the two-phase partition of ions between the bulk water and the interfacial water. The migration velocity of an ionic solute becomes lower under coexistence of a co-ion which has weaker retention on the column than the ionic solute. Thus, when an aqueous solution containing ions to which an excess amount of an electrolyte has been added is injected into the polymer column and eluted with pure water, the ions having smaller distribution coefficient or weaker retention than the added co-ion are stacked at the leading edge of the added electrolyte zone, while the other ions having larger distribution coefficient are accumulated at the trailing edge. Consequently, this ion stacking phenomenon cannot be interpreted with size-exclusion but with partition mechanism and it takes place due to the effect of the background co-ion in the eluant and/or sample solution on the distribution of the ions between the bulk water and the water incorporated in the packing material, which acts as the stationary phase.

The author examined the ion stacking phenomenon using a non-ionic porous hydrophobic material, C18 bonded silica. The distribution coefficients of conventional inorganic anions were measured on a Capcell Pak C18 UG80 column (C18 bonded silica, 5 μm , 150 \times 4.6 mm) with two different electrolyte systems. The order of the distribution coefficient between the bulk water and the interfacial water on the surface of the C18 bonded silica was $\text{IO}_3^- < \text{Cl}^- < \text{Br}^- < \text{NO}_3^- < \text{I}^- < \text{ClO}_4^- < \text{SCN}^-$. When the mixed solution containing equimolar KIO_3 and KClO_4 was introduced into the C18 column that had been equilibrated with pure water, a sharp peak of IO_3^- was detected at the front edge of eluting zone of KIO_3 . Since ClO_4^- has no UV absorbance, the peak was identified as IO_3^- of which the concentration was larger than the initial concentration. On the other hand, when a solution containing equimolar KCl and KSCN was introduced in a similar manner to the mixture of KIO_3 and KClO_4 , a stacked zone of KSCN was obtained at the trailing edge of KSCN zone, contrary to the elution profile of KIO_3 in a mixture with KClO_4 . These results indicate that, as predicted by the ion partition model, the ions having smaller distribution coefficient than the co-existing co-ion are stacked at the leading edge of the elution zone, while the other ions having larger distribution coefficients are accumulated at the trailing edge.

In order to obtain high enrichment factor using this phenomenon, the column which offers high retention capacity with remarkable selectivity should be chosen. Since hydrophilic polymer gels contain a large quantity of water compared with the hydrophobic materials and the ion partition model has been demonstrated to well interpret the separation mechanism of ionic solutes on hydrophilic polymer gel columns as well, it is expected that ion enrichment will take place regardless of the hydrophobicity and hydrophilicity of the packing material.

As most of the hydrophilic gels contain some fixed anionic charges on their surfaces, usually carboxyl groups, thus these hydrophilic gel materials may show ion-exclusion effect for anionic solutes when the ionic strength of the elu-

ant is small. It is thus expected that we are able to develop an effective ion enrichment method using combination of the ion-exclusion effect and the ion partition mechanism. The concentration of the added electrolyte was set so as to completely suppress the ion-exclusion effect and perform the separation exclusively on the basis of the partition. It was found that, on a TSKgel G2500PW_{XL} column (cross-linked hydroxylated methacrylic polymer gel, 6 μm , 300 mm \times 7.8 mm), the retention volumes of NO_3^- , I^- and SCN^- increase with increase in the ionic strength of the eluant and reach the respective constant values at the ionic strength of 50 mmol/L or above, while at 1 mmol/L or less the retention volumes of these ions converge at the interstitial volume.

Thus, a mixed solution of 1 $\mu\text{mol/L}$ of NaNO_3 and 0.1 mol/L NaCl or Na_2SO_4 was injected into the column. Very sharp peak for NO_3^- was found at the trailing edge of the added electrolyte zone. Just after injection, NaNO_3 migrates slowly under the effect of NaCl or Na_2SO_4 . When it moves out from the zone of NaCl or Na_2SO_4 , ion-exclusion effect accelerates the migration velocity of NO_3^- as the concentration of NO_3^- is very low and there is no background electrolyte. Similarly, 1 $\mu\text{mol/L}$ of I^- and SCN^- were enriched from a mixture of 0.1 mol/L NaCl or Na_2SO_4 solution when the elution was carried out with water. Experimentally it was demonstrated that there is a linear relationship between the sample volume and the enrichment factor. This means that higher enrichment can be obtained using large injection volume.

The developed on-line PIEC ion stacking method was applied to the analysis of trace impurity anions in reagent grade salts, NaCl and Na_2SO_4 . NO_2^- and NO_3^- 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. Since pure water is used as the eluant for the PIEC ion stacking, extremely low background level can be achieved by the developed method. The detection limits for nitrite and nitrate in the analysis of reagent grade Na_2SO_4 were 0.02 $\mu\text{g L}^{-1}$ and 0.04 $\mu\text{g L}^{-1}$, respectively. The contents of impurity nitrite and nitrate in the GR and EP grade salts of Na_2SO_4 calculated as the mass percentage of NaNO_2 and NaNO_3 in the sodium salt samples were 0.66-1.39 mg/kg. These impurities were found to be within the limits specified by the manufacturers.

In this thesis, 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 has also been demonstrated effective for determination of impurity anions 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. Further studies are needed to more fully explore new capability of the developed method to broaden the range of applications.

論文の審査結果の要旨

The candidate presents in her dissertation a contribution to the elucidation of ion enrichment phenomena observed in liquid chromatographic processes with aqueous mobile phase on the columns packed with non-ionic porous materials. Separations and enrichments of ionic solutes in aqueous solutions are usually achieved using materials carrying fixed charges. On the other hand, it has been reported that non-ionic or electrically neutral materials are also capable of separating ions in an aqueous solution without any additives such as ionic surfactants for ion pair formation. As well as the separation, an interesting finding of ion enrichment has recently been reported. That is, it has been found out that an enhancement of the concentrations of the component ions in the sample solution containing two different electrolytes takes place when the solution is introduced into a column packed with a nanoporous material and eluted with pure water. However, the mechanism of the ion enrichment phenomenon had not been clarified. The candidate succeeded in interpreting the mechanism of the ion enrichment phenomenon in terms of a rational physicochemical model, the ion partition model. Furthermore, she developed a simple and versatile method for anion enrichment using ion exclusion effect of fixed anionic charges on a packing material as well as the ion stacking by partition, and successfully applied the method to the ion chromatographic analysis of trace anions in water and salt samples.

The thesis consists of five chapters. Chapter 1 constitutes the general introduction and describes the overview of the scientific background of research. Chapter 2 explains the theoretical model for the distribution of ions in partition chromatography and related mathematical expressions. Chapter 3 demonstrates experimental verification of the ion partition model for the interpretation of ion enrichment on the columns packed with hydrophobic and hydrophilic materials. Chapter 4 presents a novel ion chromatographic method for the determination of trace anions in water and salt samples developed on the basis of the ion enrichment phenomenon in partition chromatographic process, partition/ion-exclusion chromatographic ion stacking-ion chromatography (PIEC ion stacking-IC). Chapter 5 contains the general conclusions of the study and the future perspectives.

Various mechanisms based on electrostatic interactions such as ion-exchange, ion exclusion, and ion pair formation are involved in the separation processes of ionic solutes in aqueous solutions. However, the majority of separations of inorganic ions are dominated by ion-exchange reactions, where the separation is due to the difference in affinity of different ions for the fixed ionic groups on the material. On the other hand, it has been reported that non-ionic materials can also separate not only of organic ions but also of simple inorganic ions in an aqueous solution. 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 the 1960s. It has been demonstrated that the separation of ionic compounds observed not only on hydrophilic polymer gel columns but also on the columns packed with hydrophobic materials can be interpreted successfully with the partition between the bulk water and the water incorporated in the polymer matrices on the basis of a stoichiometric ion partition model.

As well as the separation, an interesting finding of ion enrichment has recently been reported. It was found out that an enhancement of the concentrations of the component ions in the sample solution containing two different electrolytes takes place when the solution is introduced to a column packed with nanoporous materials and eluted with pure water. The candidate 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 ob-

served for any materials that incorporate water in their matrices. Thus, she applied the ion partition model to the explanation of the ion enrichment phenomena observed in liquid chromatography with nonionic polymer gel columns. It has been shown in Chapters 2 and 3 that the ion enrichment in liquid chromatographic processes can indeed be interpreted in terms of the ion partition model. 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 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.

Furthermore, the candidate developed a simple and versatile method, partition/ion-exclusion chromatographic ion stacking (PIEC stacking), for effective enrichment of anionic solutes in aqueous solutions 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 candidate demonstrated in Chapter 3 that one could achieve the enrichment factor as high as several hundred for some inorganic anions with a hydrophilic polymer column of 300 mm length.

The determination of trace-level anions in the presence of a large excess of matrix interferences is a common analytical problem. Although a lot of spectrophotometric and electrochemical methods have been reported for the measurement of some inorganic anions, ion chromatography is perhaps the most accepted technique for monitoring inorganic anions in water samples. Nevertheless, there is a need to find effective and fast preconcentration techniques for increasing the sensitivity of detection because it is often difficult to detect low level inorganic anions in the presence of a large excess of foreign ions directly by spectrophotometric and conductivity measurements. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are widely used as techniques for the separation and preconcentration of both organic and inorganic analytes from aqueous samples. In particular, integrated on-line SPE-LC column switching techniques have become popular for various analytical applications. With this technique, the analyte ions are stripped from the sample solution onto an SPE concentrator column, and then eluted from the column with a suitable eluant to an analytical column. However, they have several drawbacks, such as the use of organic solvents and/or toxic reagents for the extraction or elution of the analyte ions, which make them expensive, tedious, and time consuming. In addition, the loading and elution efficiencies of the analyte should be high enough so as to allow accurate and precise determinations. On-column concentration may be one of the methods that can minimize the loss of the analytes in the preconcentration process. However, very few, if any, effective and versatile methods for the on-column concentration of trace anions have been reported.

The candidate expected that the hyphenation of PIEC stacking and ion chromatography (IC) should provide a powerful method for the determination of trace amounts of analyte anions in water samples. 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 sample 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, which will lead to the enrichment of anions. Based on these considerations, the candidate achieved a new analytical methodology, on-line PIEC stacking-IC, for the determination of trace inorganic anions in water and salt samples. 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 a 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. The developed on-line PIEC ion stacking-IC method was successfully applied to the determination of nitrate ion in tap water and impurity nitrite and nitrate in reagent-grade sodium salts, sodium chloride and sodium sulfate.

The candidate clearly demonstrates creative abilities in her research field, and the thesis makes an original contribution to the knowledge and understanding of separation science and analytical chemistry. Chapters 2 and 3 comprise an article published in *Journal of Separation Science* (2017) and Chapter 4 consists of an article in *Analytical Sciences* (2018). The successful publication of the results in these international scientific journals bears witness of the originality of contributions of her PhD studies. The thesis as a whole meets the required standard of a doctoral thesis to justify the award of a PhD.