Sample Pretreatment Using Chitosan-based Chelating Resin for the Determination of Trace Metals in Seawater Samples by Inductively Coupled Plasma-Mass Spectrometry

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A sample-pretreatment method using a chitosan-based chelating resin, ethylenediamine-*N,N,N'*-triacetate-type chitosan (EDTriA-type chitosan), was developed for the preconcentration of trace metals in seawater and separation of the seawater matrix prior to their determination by inductively coupled plasma-mass spectrometry (ICP-MS). The resin showed very good adsorption for transition metals and rare-earth elements without any interference from alkali and alkaline-earth metals in an acidic media. In the proposed method, an aliquot of a 50-mL sample at pH 4 was used. Metals adsorbed on the resin were eluted using 10 mL of 1 M nitric and measured by ICP-MS. The method was evaluated by the analysis of NASS-5 seawater reference materials for trace metals. Good agreement was obtained for most metals, which indicates that by the proposed pretreatment using the synthesized resin, seawater samples can be favorably measured by ICP-MS.

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Trace metals in aquatic systems have become a significant topic of concern in various fields associated with water quality, as well as a concern of the general public. Among several kinds of water samples, the determination of trace metals in seawater is the most interesting challenge in analytical chemistry, because of high concentrations of matrix and very low concentrations of analytes. Although inductively coupled plasma-mass spectrometry (ICP-MS) provides most accurate, sensitive and reliable detection for trace metals, its low tolerance of dissolved solids prohibits the direct analysis of trace metals in seawater samples by using this instrument. In the determination of trace metals in seawater by ICP-MS, the transition metals are interfered by polyatomic ions derived from alkali and alkaline-earth metals, such as oxide and hydroxide.¹⁻³ Therefore, preconcentration and matrix separation are required prior to the determination of trace metals by ICP-MS.

Sample pretreatment for the collection/concentration of analytes and the removal of matrix and interfering substances using chelating resin, such as commercially available iminodiacetate chelating resin, known as Chelex-100,^{4,5} Muromac A-16-9 and Toyopearl AF Chelate 650M,¹⁰⁻¹² have been used for the determination of transition metals or rare-earth elements in seawater by ICP. The resins, however, can separate only some parts of alkali and alkaline-earth metals from analytes, and such sample matrix as Na⁺, Ca²⁺, Mg²⁺ and Ba²⁺ can remain with analytes and interfere with the determination of trace metals.

Several kinds of chelating resins have been proposed for the determination of transition metals or rare-earth elements in

seawater samples.¹³⁻¹⁸ Since ICP-MS enables multi-element detection simultaneously, it is desirable to determine as many trace elements as possible, including transition metals and rare-earth elements, for a comprehensive study of the distribution of trace elements in a marine environment.

Among the chelating reagents, ethylenediamine tetraacetic acid (EDTA) is one of the most stable chelating reagents, and can form chelates with a number of trace metal ions. Therefore, EDTA is a suitable chelating moiety for a multianalyte adsorption. Some chelating resins that possess the EDTA moiety have been synthesized using several kinds of polymer matrixes, such as cellulose,¹⁹ glycidyl methacrylate divinylbenzene,²⁰ or chitosan.²¹⁻²³ Nowadays, a chelating resin that possesses the EDTA moiety, Nobias Chelate-PA1, has been commercially available (Hitachi High Technologies, Tokyo, Japan). The resin has a good adsorption ability of transition and rare-earth metals, and can separate them from alkali and alkaline-earth metals. However, a high concentration of nitric acid was necessary to recover analytes from the resin, acetone was necessary for cleaning the resin, and furthermore resin particles were very small, which makes it difficult to use in a batchwise or a column pretreatment method, because of the slow flow rate.

We are mainly interested in a sorbent based on chitosan because of its unique combination of properties, like biodegradability, biocompatibility and bioactivity, in addition to attractive physical and mechanical properties. It is also reported that chitosan has the highest chelating ability compared to other natural polymers obtained from seafood wastes, activated sludge, and synthetic polymer poly(4-aminostyrene), which is used in commercial chelating ion-exchange resins.²⁴ Since chitosan was soluble in acidic media, partial crosslinking of

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chitosan by di/polyfunctional reagents should be carried out to enable it to be used for metal complexation in acidic media.²⁵

In this study, similar functional group with EDTA, ethylenediamine triacetic acid (EDTriA) was used as a moiety to make a new derivatization of a chitosan-based chelating resin, ethylenediamine-*N,N,N'*-triacetate-type chitosan (EDTriA-type chitosan) resin. About 26 elements (Al, Mn, Co, Ni, Cu, Ag, Cd, Pb, Th, U, Y and lanthanides) can be adsorbed quantitatively on the resin (more than 95%), and can be separated from alkali and alkaline-earth metals in acidic media. Therefore, the resin can be favorably used for multi-element collection/preconcentration of trace metals and for removing the matrix in seawater before measuring trace metals by ICP-MS.

Experimental

Reagents and chemicals

Chitosan (deacetylated degree 80%) used in this work was a flake type, purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). All other reagents used for the synthesis of resin were of analytical reagent grade.

A multi-element stock standard solution (55 elements, 1 $\mu\text{g mL}^{-1}$) was prepared by diluting an analytical multi-element standard solution, XSTC-13, which contained 10 $\mu\text{g mL}^{-1}$ of metal ions (Li, Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, In, Cs, Ba, Hg, Tl, Pb, Bi, Th, and U), an analytical multi-element standard solution, XSTC-1, containing 10 $\mu\text{g mL}^{-1}$ of metal ions (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Tm, Yb, and Y) purchased from Spex CertiPrep Inc. (Metuchen, NJ), and standard solutions for a single element (1000 $\mu\text{g mL}^{-1}$) for AAS from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan) (Zr, Pd, Sn, Sb, Te, Hf, Pt, and Au) in 0.1 M of a nitric acid solution. Working standard solutions were prepared daily by diluting the stock standard solution with nitric acid. All dilution procedures were performed by weight.

Ultra-pure grade nitric acid (60%, density of 1.38 g mL^{-1} ; Kanto Chemicals, Tokyo, Japan), was used and diluted with an ultra-pure water to give a 0.01 M, or a 1 M solution.

An internal standard solution was prepared by diluting a rhodium and a germanium standard solution for AAS from Wako Pure Chemical Industries, Ltd. (Japan) in 1 M nitric acid. These solutions were used to compensate for any instrumental drift or physical interferences. An internal standard was added to blanks, samples, and standards in a similar manner, so that dilution effects resulting from the addition could be disregarded.^{26,27} An appropriate volume of an internal standard was added to reach a concentration of 10 ng mL^{-1} in the final solution.

A stock solution of 4 M ammonium acetate buffer solution was prepared by mixing appropriate amounts of concentrated acetic acid and a concentrated ammonia solution, and diluted with ultra-pure water. Both the acetic acid and the ammonia solution were of electronic industrial reagent grade from Kanto Chemicals (Japan). Buffer solutions of 0.2 and 0.5 M ammonium acetate were prepared by diluting a 4 M ammonium acetate stock standard solution and adjusting to pH 3 – 9 with small amounts of concentrated ammonia or acetic acid.

The ultrapure water (18.3 $\text{M}\Omega\text{ cm}^{-1}$ resistivity) was prepared by an Elix-3/Milli-Q element system, Nihon Millipore (Tokyo, Japan).

An artificial seawater sample containing 0.47 M Na^+ , 0.05 M Mg^{2+} , 0.01 M Ca^{2+} , 0.01 M K^+ , 0.55 M Cl^- and 0.03 M SO_4^{2-} was prepared by mixing appropriate amounts of salts of calcium

Table 1 ICP-MS operating conditions

ICP-MS	Model Seiko SPQ 8000 H quadrupole type
Plasma condition	
Forward power	1.1 kW
Reflected power	< 5 W
Plasma gas	Ar 15 L min^{-1}
Carrier gas	Ar 0.45 L min^{-1}
Auxiliary gas	Ar 0.50 L min^{-1}
Sample uptake rate	1.0 ml min^{-1}
Interface condition	
Sampling depth	10 mm from load coil
Sampling cone	Copper 1.1 mm i.d.
Skimmer cone	Copper 0.35 mm i.d.

chloride, sodium sulfate, potassium chloride, sodium chloride and magnesium chloride in 0.01 M nitric acid solution.^{28,29} All of the reagents were of analytical reagent grade from Wako Pure Chemical Industries, Ltd. (Japan).

Seawater samples were collected at Shibukawa seashore (Tamano City), Ushimado seashore (Setouchi City), and at Okayama Port (Okayama City). The sample solutions were acidified to pH <2 by adding concentrated nitric acid and filtered through a 0.45- μm membrane filter from Toyo Roshi Kaisha, Ltd. (Tokyo, Japan) before storing.⁴ The pH of 50 mL sample solutions was adjusted to 4 just before a column pretreatment by adding small amounts of a concentrated ammonia solution.

NASS-5 seawater reference material for trace metals was purchased from Chemical Metrology, Institute for National Measurement Standards, National Research Council of Canada (Ontario, Canada).

Apparatus

Measurements were carried out on an ICP-MS system, a Model SPQ8000H System, Seiko Instruments Co. (Tokyo, Japan). The operating conditions are listed in Table 1. Infrared spectra (4000 – 400 cm^{-1}) were recorded on a Jasco FT/IR-4100, Jasco International Co. Ltd. (Tokyo, Japan).

Procedure for synthesis of EDTriA-type chitosan resin

EDTriA-type chitosan resin was synthesized in three steps, as shown in Fig. 1.

Step 1: synthesis of cross-linked chitosan.

In this step, chitosan (flake type, $\phi 100 - 300\ \mu\text{m}$) was crosslinked using ethylene glycol diglycidyl ether (EDGE), as described in previous work.²⁵ This cross-linked chitosan was used to make the derivative resin.

Step 2: synthesis of ethylenediamine-type chitosan.

The cross-linked chitosan was reacted with chloromethoxyrane (as a coupling arm) before being coupled with ethylenediamine to perform ethylenediamine-type chitosan. The procedure was described in a previous paper.³⁰

Step 3: synthesis of EDTriA-type chitosan.

The EDTriA-type chitosan resin was synthesized by modifying ethylenediamine-type chitosan³⁰ using bromoacetic acid, as follows. Bromoacetic acid, 3.9 g (28 mmol) was dissolved in 16 mL of ice-cold water. To this solution, under cooling in an ice-water bath, was added 2.5 M NaOH until the pH reached 12. Three grams of ethylenediamine-type chitosan resin (7 mmol) was suspended in 20 mL of water and mixed with a solution of bromoacetic acid, and then warmed to 40 – 45°C in a water bath. The pH was maintained at 12 by adding a small amount of 2.5 M NaOH for 6 – 7 h. The solid product

Step 1: synthesis of cross-linked chitosan

Step 2: synthesis of ethylenediamine-type chitosan

Step 3: synthesis of EDTriA-type chitosan

Fig. 1 Reaction scheme for the synthesis of ethylenediamine-*N,N,N'*-triacetate-type chitosan (EDTriA-type chitosan) resin.

(EDTriA-type chitosan resin) was filtered on a filter paper, and then washed three times with ethanol and water. By this reaction, acetyl groups from bromoacetic acid could bind with the amino groups³¹ of the ethylenediamine-type chitosan.

Mini-column procedure for collection and concentration of trace metals

Before using, the EDTriA-type chitosan resin was cleaned to remove any metal impurities in the resin. Ten milliliters of wet resin were transferred to a plastic beaker; to it, 50 mL of 2 M nitric acid was added. The mixture was carefully stirred at low speed for 6 h. The aqueous solution was decanted, and the resin was rinsed with ultrapure water until the pH of the effluent was the same as that of ultrapure water.

Approximately 1 mL of wet resin was packed in a polypropylene Muromac® mini column, 5 mm i.d. × 50 mm, Muromachi Kagaku Kogyo Kaisha, Ltd. (Tokyo, Japan). Through the column were passed 10 mL of 1 M nitric acid, 10 mL of ultrapure water for washing, and 5 mL of a 0.5 M ammonium acetate buffer solution at pH 4 for column conditioning. After pretreatment of the column, 50 mL of sample solutions at pH 4 were passed through the column, and then 5 mL of a 0.2 M ammonium acetate buffer solution at pH 4 and 5 mL of ultrapure water were passed for eliminating the matrix. Finally, 10 mL of 1 M nitric acid was passed through the column for recovering metals adsorbed on the resin. Therefore 5-fold preconcentration was achieved. The eluent was collected in a pre-cleaned screw-capped polypropylene bottle, and then analyzed by ICP-MS after adding Rh and Ge as internal standards.

For examining the adsorption behavior of the resin, 9 mini columns containing the EDTriA-type chitosan resin were used;

each column was used for a different pH. Nitric acid for pH 1–2 and 0.5 M ammonium acetate buffer for 3 < pH < 9 were used for column conditioning, while a lower concentration of a buffer solution, 0.2 M ammonium acetate buffer, were used to remove the sample matrix. The procedure was carried out in the same manner as describe earlier. For the sample, 10 mL of a solution containing 5 ng mL⁻¹ of each element, the pHs of which were adjusted to appropriate values, was used. All of the effluents were collected and analyzed by ICP-MS after adding Rh and Ge as internal standards.

Throughout the column pretreatment, the flow rate (by gravity) of the rinsing solutions, samples, and the eluents was about 2 mL min⁻¹. The time required for the whole column pretreatment, for 50 mL of sample solutions, was 48 min.

Acid digestion prior to the analysis of Al, Mn, Co, and Ni

For the determination of Al, Mn, Co, and Ni, a seawater sample should be digested prior to analysis. To 50 mL of a seawater sample placed in a PTFE beaker, 1 mL of (1 + 1) nitric acid and 0.5 mL of (1 + 1) hydrochloric acid were added. The beaker was placed in an evaporation chamber for solution evaporation at a temperature of approximately, but not higher than at 85°C. The volume of the sample aliquot was reduced to about 3 mL by gentle heating at 85°C, and refluxed for 30 min by increasing the temperature at 120°C, and then evaporated to dryness.³² The evaporation chamber was covered with a clear polyvinyl sheet to prevent samples from contamination. The residue in the beaker was redissolved with 10 mL of 0.01 M nitric acid and adjusted to pH 4 by adding small amounts of a concentrated ammonium solution before passing through a column that contained EDTriA-type chitosan resin. Elements adsorb on the resin were eluted by 10 mL 1 M nitric acid and

measured by ICP-MS.

Since the concentrations of Al and Mn in real seawater samples were sufficiently high to be detected by ICP-MS without any preconcentration, for the determination of Al and Mn, 10 mL of samples was used. To a 10-mL seawater sample placed in a PTFE beaker, 0.2 mL of (1 + 1) nitric acid and 0.1 mL of (1 + 1) hydrochloric acid were added, and then evaporated in the same manner as in the previous procedure.

Results and Discussion

Characteristics of the EDTriA-type chitosan resin

Infrared spectra (4000 – 400 cm^{-1}) were recorded (Fig. 2). In the fingerprint region (1500 – 500 cm^{-1}), the spectrum of EDTriA-type chitosan resin was totally different from that of the cross-linked chitosan and the ethylenediamine-type chitosan.

New bands appeared when a carboxymethyl group connected to the ethylenediamine-type chitosan resin. The band at 680 cm^{-1} in Fig. 2C confirmed a O=C=O bend of the carboxylate group. Other bands at 1100 and 1300 cm^{-1} confirmed C–O stretch, and at 2650 – 2750 cm^{-1} confirmed O–H stretch of carboxylic acid.³³ The band at 1550 cm^{-1} , which confirmed a N–H bend of 1° amine of the cross-linked chitosan and ethylenediamine-type chitosan (Figs. 2A and 2B), disappeared in the spectrum of EDTriA-type chitosan (Fig. 2C), which means that almost all of the free amine was converted to the aminoacetate.

Adsorption behavior of elements on EDTriA-type chitosan resin

The adsorption behavior of elements on EDTriA-type chitosan resin was examined using 10 mL of 5 ng mL^{-1} standard metal solutions. As shown in Fig. 3, the EDTriA-type chitosan resin has very good adsorption ability for transition metals and rare-earth elements from pH 3 to 7. The resin did not adsorb both alkali and alkaline-earth metals in acidic media (pH <6). Increasing the pH could increase the adsorption of alkaline-earth metal on the resin. Therefore, pH 4 was selected for further investigation. All elements that could adsorb on the resin were found to be recovered at greater than 95% with 10 mL of 1 M nitric acid.

Compared with the ethylenediamine-type chitosan resin which can selectively adsorb silver from pH 1 to 8, as reported in a previous paper,³⁰ the proposed resin, synthesized by introducing an acetyl group to the amino group of the ethylenediamine-type chitosan, EDTriA-type chitosan resin, has a totally different adsorption behavior. There are 26 elements that can be adsorbed with greater than 95% on the EDTriA-type chitosan resin, including silver. This means that introducing the acetyl group into the resin not only gives additional chelating rings, but can also change the resin character. Similarly to the chelate in aqueous solutions, the selectivity of suitable functional groups fixed into the polymer had also been explained on the basis of the concept of strong and weak acids and bases³⁴ or hard and soft ligands.³⁵ Transforming donor atoms (N, N) of ethylenediamine-type chitosan to donor atoms (O, N) of EDTriA-type chitosan could increase the adsorption ability of the EDTriA-type chitosan toward such “borderline” cations as transition metals.

Adsorption capacity of EDTriA-type chitosan resin

Though a number of metals can adsorb on the resin, Cu(II) was selected for examining the adsorption capacity of the EDTriA-type chitosan resin. One milliliter of the wet EDTriA-type chitosan resin was equilibrated in 100 mL of 1.4 mM Cu(II) ion at pH 4. The adsorption capacity of Cu(II) on the EDTriA-type chitosan resin was 0.043 mmol mL^{-1} of the resin

Fig. 2 FTIR spectra: A, cross-linked chitosan; B, ethylenediamine-type chitosan; C, EDTriA-type chitosan.

or 0.12 mmol g^{-1} of the resin, and the $t_{1/2}$ of Cu(II) adsorbed was less than 10 min.

Collection efficiency in column pretreatment

The collection efficiencies of metals on a column packed with EDTriA-type chitosan resin was studied by comparing the slopes of the calibration graph, which were obtained by column pretreatments and direct measurements. In a direct measurement, the standard solution in 1 M HNO_3 was measured directly by ICP-MS, without passing it through the column. The result is given in Table 2.

There were 26 metals that could be collected with greater than 95% efficiency on a column packed with EDTriA-type chitosan resin when 10 mL of the sample was used, as shown in Fig. 3. However, when 50 mL of the sample was used, as given in Table 2, the collection of Al and Mn decreased to about 90%. Since the concentrations of Al and Mn in real samples were high enough to be detected without any preconcentration, the preconcentration procedure was not performed. For determining Al and Mn, mini column procedure was used only for removing the seawater matrix. For Ag, a calibration graph obtained by the column pretreatment was not linear; the chelation of Ag with the resin seemed to be weak.

Effect of major cations in the seawater matrix on the determination of trace metals

Interference arising from the salt matrix present in the samples could vary with the sample salinity, and therefore could not be corrected by using a blank solution. Since major cations present in seawater were alkali and alkaline-earth metals (Mg, Ca, Na, and K), and both alkali and alkaline-earth metals in acidic media did not adsorb on the EDTriA-type chitosan resin, and could be passed to waste while the trace metals were retained on the resin, trace metals could be separated from the major cations in the seawater matrix. This means that the effect of major cations could be eliminated by a column pretreatment using EDTriA-type chitosan resin.

Effect of anions in the seawater matrix on the determination of trace metals

The effect of major anions, chloride and sulfate, in the sample matrix was studied by comparing the slopes of the calibration

Fig. 3 Adsorption behavior of EDTriA-type chitosan for metals at various pH values. Sample volume, 10 mL; concentration of each metal, 5 ng mL⁻¹; eluent, 10 mL of 1 M nitric acid.

Table 2 Slopes ratio (*r*) of the calibration graph obtained by a column pretreatment to that obtained by a direct measurement

Element	Slope ratio (<i>r</i>), HNO ₃ (column)/ HNO ₃ (direct)	Slope ratio (<i>r</i>), artificial seawater (column)/HNO ₃ (direct)	
		Without acid digestion	With acid digestion
Al	0.8948	0.8149 ^a	0.9317 ^a
Mn	0.9063	0.7530 ^a	0.9856 ^a
Co	1.0053	0.9795	
Ni	0.9471	0.9898	
Cu	0.9600	0.9123	
Cd	0.9598	0.9205	
In	1.0007	0.9810	
Pb	0.9359	0.9731	
Th	0.9381	0.9108	
U	1.0192	1.0207	
Y	0.9756	0.9981	
La	0.9992	0.9739	
Ce	0.9870	1.0389	
Pr	0.9827	0.9921	
Nd	1.0420	0.9730	
Sm	0.9854	0.9066	
Eu	0.9733	1.0318	
Gd	0.9579	1.0530	
Tb	0.9896	0.9709	
Dy	1.0444	1.0124	
Ho	0.9790	0.9988	
Er	0.8936	0.9779	
Tm	1.0027	0.9855	
Yb	1.0171	1.0780	
Lu	0.9980	1.0186	

a. Sample volume, 10 mL; eluent, 10 mL (1-fold preconcentration).

graphs for artificial seawater samples, which could be obtained by a column pretreatment and calibration graphs for standard samples in 1 M HNO₃, which could be obtained by direct measurements. The results given in Table 2 indicate that both of the calibration graphs for metals are in good agreement with one another (slope ratio was about 1), except for Al and Mn. This means that the major anions in the seawater matrix did not

interfere with the collection of trace metals on the column that contained EDTriA-type chitosan resin, except for Al and Mn.

Aluminum forms stable complexes with sulfate and electron-rich species, such as fluoride and chloride, which make it difficult to bind with the resin. Additionally, Al may be bound to organic and inorganic ligands as well as to particulate matter.³⁶ From thermodynamic considerations, MnO₂ is expected to be the stable form of Mn in seawater. However, seawater contains a significant quantity of dissolved Mn(II), which is slowly oxidized. Investigations of the oxidation of Mn(II) have shown that the process is autocatalytic, and depends on the reaction conditions.^{4,36} Only Mn(II) can form a stable chelate with EDTriA-type chitosan resin.

To solve the problem, before passing through the column, an artificial seawater samples was spiked with several concentrations of Al and Mn, and was then digested.³² From the result shown in Table 2, the slope ratio of the calibration graphs for metals in the digested artificial seawater sample and those in the standard samples were increased to 0.93 and 0.99 for Al and Mn, respectively. This means that by digestion using HNO₃ and HCl, all binding of Al with other compound could be completely destroyed and all Mn was reduced to Mn(II); therefore, Al and Mn could be collected completely on the resin.

Effect of other compounds in the seawater matrix on the determination of trace metals

Since artificial seawater was made by mixing only major cations and anions,^{28,29} real seawater samples have to be used to study the effect of other compounds that exist in seawater samples, such as natural or anthropogenic ligands. In this study, the seawater sample used was collected at the Shibukawa seashore, Tamano City, Okayama Prefecture. Shibukawa seawater was digested,³² and the analytical results from both sample preparation methods, the digested and the undigested procedure, were compared. As shown in Table 3, without acid digestion, only 50–80% of Al, Mn, Co, and Ni were collected

Table 3 Result obtained for trace metals in Shibukawa seawater without and with acid digestion

Element	Without acid digestion/ ng mL ⁻¹	With acid digestion ^b / ng mL ⁻¹
Al ^a	13	27
Mn ^a	7.2	10.0
Co	0.069	0.087
Ni	0.92	1.35
Cu	2.6	2.4
Cd	0.17	0.17
In	0.0012	0.0011
Pb	0.22	0.20
Th	0.0016	0.0016
U	2.6	2.5
Y	0.070	0.066
La	0.058	0.056
Ce	0.091	0.087
Pr	0.011	0.011
Nd	0.043	0.041
Sm	0.0077	0.0071
Eu	0.00084	0.00076
Gd	0.0107	0.0097
Tb	0.0016	0.0015
Dy	0.010	0.0090
Ho	0.0021	0.0021
Er	0.0074	0.0065
Tm	0.00049	0.00044
Yb	0.0069	0.0068
Lu	0.00099	0.00088

a. Sample volume, 10 mL; eluent, 10 mL (1-fold preconcentration).

b. Before column pretreatment, samples were digested using HNO₃ and HCl, based on EPA Method 200.8.³²

on the resin, while for other metals no significant differences in the signal were obtained between undigested samples and the digested one.

In aqueous solutions Co exists mainly in the II oxidation state, but in some complexes Co(II) is readily oxidizable to Co(III). In certain circumstances, anthropogenic Co(III) picolinate, and to a lesser extent, naturally occurring cobalamine may also be present.^{36,37} Some complex species are sufficiently stable to persist in the environment for considerable periods of time, and thus influence the adsorption behavior of Co on a column containing EDTriA-type chitosan resin.

In natural water the dissolved forms of Ni can include the hydrated Ni²⁺ cation, complexes with inorganic ligands, such as chloro- and carbonato-complexes, or with various anthropogenic and natural ligands, such as terrestrial humic substances, or phytoplankton metabolites.^{36,38} These ligands compete with EDTriA-type chitosan resin to form stable complexes with Ni.

As shown in Table 2, the collection of Co and Ni in artificial seawater on a column containing EDTriA-type chitosan resin was higher than 98%. This means that the lower adsorption ability of Co and Ni in real seawater samples did not come from chloride and sulfate, but from other compounds or ligands.^{37,39} For complete adsorption of Co and Ni on the column contained EDTriA-type chitosan resin, before the column pretreatment, samples were digested to destruct all binding of Co and Ni with other compounds or ligands.

Blank and detection limit

Blank values, method detection limits (MDL), and instrument detection limits (IDL) are given in Table 4. An aliquot containing 50 mL of 0.01 M nitric acid, the pH of which was adjusted to 4 just before a column pretreatment by adding small amounts of ammonia solution, was used as a blank solution and was processed in the same manner as the sample solutions. The average of 7 replicates was reported as blank values.

MDL was based on blank + 3SD, calculated as blank values, and three-times the standard deviation of the blank values.^{40,41} IDL is the concentration equivalent to the analyte signal, which

Table 4 Blank values, method detection limits (MDL), and instrument detection limits (IDL)

Element	Blank ^a /ng mL ⁻¹	MDL ^b /ng mL ⁻¹	IDL ^c /ng mL ⁻¹
²⁷ Al	0.040	0.070	0.16
⁵⁵ Mn	0.040	0.10	0.24
⁵⁹ Co	0.0011	0.0020	0.0078
⁶⁰ Ni	0.047	0.054	0.031
⁶³ Cu	0.052	0.12	0.63
¹¹¹ Cd	0.0032	0.0057	0.017
¹¹³ In	0.00042	0.00059	0.0028
²⁰⁸ Pb	0.00051	0.011	0.016
²³² Th	0.0028	0.0080	0.011
²³⁸ U	0.0014	0.0046	0.0086
⁸⁹ Y	0.00040	0.00096	0.0029
¹³⁹ La	0.00039	0.0011	0.0024
¹⁴⁰ Ce	0.0023	0.0056	0.015
¹⁴¹ Pr	0.00010	0.00035	0.0019
¹⁴⁶ Nd	0.00028	0.0014	0.0078
¹⁴⁷ Sm	0.00020	0.0010	0.0071
¹⁵³ Eu	0.00011	0.00051	0.0025
¹⁵⁷ Gd	0.00018	0.00081	0.0023
¹⁵⁹ Tb	0.00014	0.00021	0.0014
¹⁶³ Dy	0.00013	0.00084	0.0045
¹⁶⁵ Ho	0.000025	0.00015	0.0013
¹⁶⁰ Er	0.00022	0.00083	0.0032
¹⁶⁰ Tm	0.000030	0.00016	0.00098
¹⁷² Yb	0.000074	0.00045	0.0032
¹⁷⁵ Lu	0.000015	0.00012	0.00095

a. Sample blank, 50 mL of 0.01 M HNO₃, pH was adjusted to 4 by adding small amount of ammonia; eluent, 10 mL 1 M HNO₃. Data were averaged for 7 replicates.

b. Method detection limit, corresponding to blank + 3SD of sample blank with 5-fold preconcentration, 7 replicates.

c. Instrument detection limit, corresponding to 3SD of 1 M HNO₃, 10 measurements.

is equal to three-times the standard deviation of 10 replicate measurements of 1 M nitric acid as the calibration blank signal (by direct measurement).³² From the obtained results, a 5-fold enrichment factor is sufficient to detect all trace metals in seawater samples.

Accuracy and precision

The accuracy and precision of the proposed column pretreatment method using the EDTriA-type chitosan resin were examined by analyzing the NASS-5 seawater reference material for trace metals issued by the National Research Council of Canada (Ottawa, Ontario, Canada). Since there are no certified values for rare-earth elements, the results obtained in this work were compared with reference values reported by Willie *et al.*¹⁰ and Zhu *et al.*⁵ The sample pH was adjusted to 4 before a column pretreatment, and used without any acid digestion to prevent the metal ions from any losses or contamination. Therefore, the results of Al, Mn, Ni and Co obtained in this experiment are lower than those of the certified and reference values, as shown in Table 5. For other elements, the results were in good agreement with the certified and the reference values.

The obtained results indicate that the present method has good accuracy and precision, which are less than 10% of the relative standard deviation (RSD), and is applicable to the multi-element preconcentration and determination of trace metals in seawater samples.

Application to the seawater samples

The proposed method was successfully applied to the determination of trace metals in seawater samples from Okayama Prefecture, Japan, collected at Shibukawa seashore (Tamano City), Ushimado seashore (Setouchi City), and at Okayama Port (Okayama City). Only for the determination of Al, Mn, Co, and Ni, samples were digested using HNO₃ and HCl before passing through a column containing EDTriA-type

Table 5 Analytical results of trace metals in the NASS-5 seawater reference material for trace metals (ng L⁻¹)

Element	This study ^a	Certified value	Reference value 1 ^b	Reference value 2 ^c
Al	0.079 ± 0.001			0.11 ± 0.02
Mn	0.51 ± 0.0	0.92 ± 0.06		0.87 ± 0.04
Co	0.0096 ± 0.0009	0.011 ± 0.003	0.015 ± 0.001	0.013 ± 0.002
Ni	0.19 ± 0.01	0.25 ± 0.03	0.28 ± 0.01	0.24 ± 0.01
Cu	0.31 ± 0.02	0.30 ± 0.05	0.26 ± 0.01	0.31 ± 0.03
Cd	0.027 ± 0.001	0.023 ± 0.003	0.019 ± 0.002	0.03 ± 0.00
In	0.0032 ± 0.0003			
Pb	0.0059 ± 0.0005	0.008 ± 0.005	0.008 ± 0.002	
Th	0.00081 ± 0.00008			0.0003 ± 0.0000
U	2.8 ± 0.1	2.6	2.6 ± 0.0	2.66 ± 0.05
Y	0.020 ± 0.001			0.02 ± 0.00
La	0.014 ± 0.001		0.013 ± 0.001	0.0111 ± 0.0005
Ce	0.0047 ± 0.0003		0.004 ± 0.001	0.0049 ± 0.0003
Pr	0.0017 ± 0.0001		0.0015 ± 0.0002	0.0019 ± 0.0001
Nd	0.0076 ± 0.0005		0.0099 ± 0.0018	0.0068 ± 0.0005
Sm	0.0041 ± 0.0002		0.004 ± 0.000	0.004 ± 0.0003
Eu	0.00028 ± 0.00002		0.00026 ± 0.00005	0.00040 ± 0.00001
Gd	0.0017 ± 0.0003		0.0015 ± 0.0003	0.0021 ± 0.0001
Tb	0.00035 ± 0.00002		0.00029 ± 0.00005	0.00024 ± 0.00000
Dy	0.0018 ± 0.0002		0.0016 ± 0.0003	0.0018 ± 0.0001
Ho	0.00037 ± 0.00004		0.00036 ± 0.00005	0.00044 ± 0.00002
Er	0.0013 ± 0.0001		0.0012 ± 0.0002	0.0013 ± 0.0001
Tm	0.00016 ± 0.00003		0.00015 ± 0.00003	0.00024 ± 0.00000
Yb	0.0012 ± 0.0001		0.0011 ± 0.0002	0.0013 ± 0.0001
Lu	0.00017 ± 0.00002		0.0002 ± 0.0000	0.00022 ± 0.00002

a. Without acid digestion: sample volume, 50 mL; eluent, 10 mL 1 M HNO₃; 3 replicates. b. Cited from Ref. 10. c. Cited from Ref. 5.

chitosan resin; 10 mL of samples were used for Al and Mn determination, while 50 mL of samples were used for Co and Ni determination. For other trace metals, 50 mL of samples were used without any acid digestion.

As shown in Table 6, the concentrations of trace metals in Ushimado seawater were generally higher than those in Okayama Port and Shibukawa seawater. Samples were spiked with several concentrations of metal ions; the spiked concentrations were as close as possible to the concentration found, and the recovery test was examined. The recovery results were in the range of 91% (Ho) to 110% (Tm).

Conclusions

A novel chelating resin using chitosan as a base material, EDTriA-type chitosan, was synthesized for the multi-element collection/concentration of trace metals. The resin had good adsorption ability for transition metals and rare-earth elements, and did not adsorb both alkali and alkaline-earth metals in acidic media. At pH 4, about 25 elements could be collected quantitatively and separated from alkali and alkaline-earth metals. All of the metals could be easily recovered from the resin using 1 M nitric acid. The resin was also stable for several months of storage, and it was found that even after about 30-time use for the pretreatment of samples, the adsorption behavior was almost the same as the first adsorption behavior.

By a pretreatment using EDTriA-type chitosan resin, trace metals including rare-earth elements in seawater could be determined by ICP-MS with only 5-fold preconcentration.

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Table 6 Analytical results of trace metals in real seawater samples

Element	Seawater at Okayama Port ^a			Seawater at Shibukawa ^a			Seawater at Ushimado ^a		
	Spiked/ ng mL ⁻¹	Found/ng mL ⁻¹	Recovery, %	Spiked/ ng mL ⁻¹	Found/ng mL ⁻¹	Recovery, %	Spiked/ ng mL ⁻¹	Found/ng mL ⁻¹	Recovery, %
Al ^{b,c}	—	23 ± 2	—	—	30 ± 2	—	—	60 ± 3	—
Mn ^{b,c}	—	8.0 ± 0.6	—	—	9.9 ± 1.5	—	—	11 ± 2	—
Co ^c	—	0.082 ± 0.005	—	—	0.087 ± 0.004	—	—	0.096 ± 0.003	—
Ni ^c	0.05	0.128 ± 0.003	92.1 ± 5.8	0.05	0.135 ± 0.003	96.7 ± 6.4	0.05	0.145 ± 0.003	97.4 ± 5.8
	—	1.1 ± 0.0	—	—	1.3 ± 0.1	—	—	1.1 ± 0.1	—
Cu	1	2.0 ± 0.1	92.3 ± 6.6	1	2.4 ± 0.0	107.5 ± 4.5	1	2.1 ± 0.9	96.0 ± 9.1
	—	1.2 ± 0.0	—	—	2.6 ± 0.0	—	—	4.1 ± 0.0	—
Cd	0.3	1.5 ± 0.0	93.4 ± 1.9	0.3	2.9 ± 0.2	103.5 ± 6.8	0.3	4.4 ± 0.1	97.9 ± 2.6
	—	0.037 ± 0.008	—	—	0.17 ± 0.02	—	—	0.091 ± 0.007	—
In	0.03	0.069 ± 0.002	108.1 ± 3.0	0.1	0.27 ± 0.01	101.2 ± 5.7	0.1	0.190 ± 0.010	99.0 ± 8.4
	—	0.0017 ± 0.0003	—	—	0.0012 ± 0.0001	—	—	0.00070 ± 0.00016	—
Pb	0.03	0.0349 ± 0.0029	110.6 ± 8.3	0.03	0.0310 ± 0.0007	99.5 ± 2.1	0.03	0.0316 ± 0.0019	102.9 ± 4.0
	—	0.56 ± 0.02	—	—	0.22 ± 0.001	—	—	1.2 ± 0.0	—
Th	0.3	0.85 ± 0.04	97.1 ± 4.8	0.1	0.31 ± 0.02	91.7 ± 4.8	0.3	1.5 ± 0.0	98.9 ± 0.1
	—	0.0030 ± 0.0006	—	—	0.0016 ± 0.0004	—	—	0.032 ± 0.003	—
U	0.03	0.0320 ± 0.0015	96.7 ± 4.7	0.03	0.0334 ± 0.0008	106.1 ± 2.3	0.03	0.064 ± 0.002	107.8 ± 3.5
	—	0.77 ± 0.02	—	—	2.6 ± 0.1	—	—	2.4 ± 0.1	—
Y	0.03	1.06 ± 0.01	97.2 ± 1.2	0.3	2.9 ± 0.1	98.6 ± 2.7	0.3	2.7 ± 0.1	95.2 ± 2.1
	—	0.078 ± 0.001	—	—	0.070 ± 0.003	—	—	0.12 ± 0.01	—
La	0.03	0.107 ± 0.001	97.5 ± 0.6	0.03	0.102 ± 0.001	105.7 ± 0.9	0.03	0.15 ± 0.00	99.8 ± 1.1
	—	0.082 ± 0.004	—	—	0.058 ± 0.003	—	—	0.15 ± 0.01	—
Ce	0.03	0.110 ± 0.002	94.7 ± 2.1	0.03	0.089 ± 0.001	103.6 ± 0.6	0.03	0.18 ± 0.00	91.9 ± 2.2
	—	0.13 ± 0.00	—	—	0.092 ± 0.001	—	—	0.24 ± 0.01	—
Pr	0.03	0.16 ± 0.00	97.8 ± 1.1	0.03	0.122 ± 0.002	100.0 ± 6.0	0.03	0.27 ± 0.01	101.8 ± 3.1
	—	0.013 ± 0.001	—	—	0.011 ± 0.001	—	—	0.030 ± 0.002	—
Nd	0.01	0.022 ± 0.001	94.8 ± 5.2	0.01	0.020 ± 0.000	95.4 ± 2.6	0.03	0.061 ± 0.001	102.2 ± 2.6
	—	0.058 ± 0.003	—	—	0.041 ± 0.001	—	—	0.12 ± 0.01	—
Sm	0.03	0.088 ± 0.001	93.8 ± 1.7	0.03	0.072 ± 0.004	100.7 ± 6.0	0.03	0.15 ± 0.00	92.4 ± 0.7
	—	0.010 ± 0.001	—	—	0.0074 ± 0.0007	—	—	0.023 ± 0.001	—
Eu	0.01	0.020 ± 0.001	93.6 ± 3.3	0.005	0.0125 ± 0.0007	103.1 ± 5.9	0.01	0.033 ± 0.002	97.2 ± 5.0
	—	0.0014 ± 0.0002	—	—	0.00082 ± 0.0002	—	—	0.0047 ± 0.0097	—
Gd	0.001	0.0025 ± 0.0002	104.2 ± 6.9	0.001	0.00172 ± 0.0002	91.0 ± 9.1	0.005	0.0092 ± 0.0071	91.0 ± 7.7
	—	0.013 ± 0.001	—	—	0.011 ± 0.002	—	—	0.026 ± 0.0015	—
Tb	0.01	0.023 ± 0.000	96.1 ± 1.3	0.01	0.020 ± 0.001	93.9 ± 5.7	0.01	0.036 ± 0.0018	99.7 ± 5.0
	—	0.0019 ± 0.0002	—	—	0.0018 ± 0.0001	—	—	0.0050 ± 0.0007	—
Dy	0.001	0.0029 ± 0.0002	104.3 ± 6.8	0.001	0.0028 ± 0.0002	95.2 ± 9.1	0.005	0.0100 ± 0.0009	99.6 ± 9.1
	—	0.0082 ± 0.0009	—	—	0.011 ± 0.001	—	—	0.024 ± 0.001	—
Ho	0.005	0.0132 ± 0.0010	99.2 ± 8.0	0.01	0.021 ± 0.001	100.8 ± 6.7	0.01	0.033 ± 0.003	97.0 ± 9.0
	—	0.0014 ± 0.0004	—	—	0.0022 ± 0.0001	—	—	0.0041 ± 0.0007	—
Er	0.001	0.0024 ± 0.0001	103.0 ± 4.0	0.001	0.0031 ± 0.0001	90.7 ± 2.3	0.005	0.0095 ± 0.0009	106.8 ± 9.4
	—	0.0054 ± 0.0009	—	—	0.0074 ± 0.0005	—	—	0.015 ± 0.001	—
Tm	0.005	0.0100 ± 0.0003	95.4 ± 2.9	0.005	0.012 ± 0.001	92.9 ± 9.9	0.01	0.024 ± 0.002	96.9 ± 6.2
	—	0.00056 ± 0.00011	—	—	0.00049 ± 0.00011	—	—	0.0018 ± 0.0001	—
Yb	0.001	0.00150 ± 0.00013	94.0 ± 8.6	0.001	0.0159 ± 0.00009	109.9 ± 6.7	0.001	0.0028 ± 0.0001	103.8 ± 3.8
	—	0.0079 ± 0.0004	—	—	0.0069 ± 0.0003	—	—	0.012 ± 0.001	—
Lu	0.005	0.0129 ± 0.0008	101.8 ± 6.2	0.005	0.0123 ± 0.0005	104.8 ± 3.9	0.01	0.022 ± 0.003	100.8 ± 9.1
	—	0.0013 ± 0.0001	—	—	0.0011 ± 0.0000	—	—	0.0012 ± 0.0001	—
	0.001	0.0024 ± 0.0001	109.4 ± 5.7	0.001	0.00211 ± 0.0001	101.9 ± 6.4	0.001	0.0022 ± 0.0002	94.0 ± 6.2

a. Sample volume, 50 mL; eluent, 10 mL (5-fold preconcentration factor); 3 replicates, except for Al and Mn. b. Sample volume, 10 mL; eluent, 10 mL (1-fold preconcentration factor); 3 replicates. c. Samples were digested using HNO₃ and HCl (based on EPA Method 200.8) before passing through the column.

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