Chemistry

Analytical Chemistry fields

Okayama University   *Year* 2007

Synthesis of novel chitosan resin derivatized with serine diacetic acid moiety and its application to on-line collection/concentration of trace elements and their determination using inductively coupled plasma-atomic emission spectrometry

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Synthesis of novel chitosan resin derivatized with serine diacetic acid moiety and its application to on-line collection/concentration of trace elements and their determination using inductively coupled plasma-atomic emission spectrometry

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Abstract

A novel chelating resin functionalized with serine diacetic acid moiety was synthesized by using chitosan as base material, and applied to the collection/concentration of trace elements in environmental water samples, followed by the determination using inductively coupled plasma-atomic emission spectrometer (ICP-AES). The synthesized resin, crosslinked chitosan serine diacetic acid (CCTS-SDA), showed good adsorption behavior toward trace amounts of Cd, Pb, Cu, Ni, V, Ga, Sc, In, and Th in a wide pH range. Additionally, rare earth elements also can be retained on the resin at neutral pH region. The adsorbed elements can be easily eluted with 1 mol L^{-1} of nitric acid, and their recoveries were found to be 90-100%. The CCTS-SDA was packed in a mini-column, which was then installed in a computer-controlled auto-pretreatment system (Auto-Pret System) for on-line

trace elements collection and determination with ICP-AES. Experimental parameters which related to the improvement of sensitivity and reproducibility were optimized. The limit of detections (LOD) for 13 elements were found to be in sub-ppb level. The proposed method with CCTS-SDA resin was successfully applied to the determination of trace elements in river water samples. The method was validated by determining a certified reference material of river water, SLRS-4.

Keywords: Chelating resin; Chitosan; Serine diacetic acid moiety; Trace elements; On-line Pretreatment; Computer control; Inductively coupled plasma-atomic emission spectrometry; SLRS-4;

1 Introduction

Heavy metal ions in natural water can bring harmful effect to human health, as well as to animal and plants in aquatic environment. Therefore, monitoring these metals in environmental water samples at trace levels is very important. Accurate analysis of various complex samples containing elements at trace levels is one of the most challenging analytical tasks. Despite the rapid development of instrumentation which opens new path to trace analysis, accurate quantitative determination of a number of elements is still impaired to a greater or lesser extent by matrix and spectral interferences [1]. Moreover, some analytical

techniques, such as flame atomic absorption spectrometry (FAAS) and inductively coupled plasma-atomic emission spectrometry, do not have enough sensitivity to directly determine certain elements in a relative clean environmental water sample, and thus a preconcentration step is required before applying them to trace metal analysis.

In recent years, the importance of separation and concentration technique involving chelating sorbent in trace analysis has risen substantially. The pretreatment of aqueous solution by sorption technique not only can increase analyte concentration to detectable level, but also can eliminate matrix effects [2].

Several materials have been proposed as a sorbent for the collection/concentration of trace elements using solid phase extraction technique, such as activated carbon [3-5], Amberlite XAD [6-12], Diaion [13], Chromosorb [14], silica [1], and alumina [15]. Activated carbon may provide economical advantage. However, physical loading of a complexing agent on activated carbon suggested that such sorbent may have less durability due to the loss of complexing agent during sorption-desorption processes. The addition of a complexing agent to each sample prior to column treatment is considered to be disadvantageous due to high reagent consumption and more organic waste will be generated [3]. The utilization of p-dimethylamino-benzaldehyde-modified nanometer SiO2 prior to ICP-AES measurement allows simultaneous determination of trace amount of Cu(II) and Pb(II) [1]; the detection limit indicates that the sensitivity of this method is not sufficient to measure Cu(II) and Pb(II)

in relatively clean environmental water. The preconcentration method using Chromosorb-103 [13] provided a better detection limit for Pb(II). However, sample pretreatment by using 8-hydroxyquinoline is required before the preconcentration procedure can be carried out.

Though the pretreatment method which utilize a sorbent mentioned above can improve sensitivity in trace metal analysis, the operation of pretreatment step is usually tedious and time consuming because it must be carried out by manual batch-wise procedure, where stringent control of the laboratory environment is required to avoid sample contamination, especially if the determination of trace level of analytes is attempted. Such drawback can be overcome by utilizing an automated on-line preconcentration procedure, where less contamination and less reagent consumption can be done [15-19].

Chitosan, an N-deacetylated product of chitin which can be obtained in large quantity from crustacean shells, has become an important natural polymer because of its unique properties, such as higher chelating ability compared to other natural polymers obtained from seafood-industries waste, easy derivatization, and biodegradability [20]. The reactive amino group in chitosan can provide easy-derivatization of this material with some chelating moiety for wider application in solid phase extraction. Compared to other synthetic polymers, chitosan possesses higher hydrophilicity thus providing faster adsorption rate in aqueous medium.

Several researchers have reported the synthesis of cross-linked chitosan to improve its

chemical durability against acid medium along with its application [21-25]. Cross-linked

chitosan itself has been derivatized with several functional groups, and has been applied to

solid phase extraction techniques; For example, cross-linked chitosan derivatized with serine

moiety for U collection [26], leucine moiety for Mo collection [27], dithiocarbamate [28] and

iminodi(methylphosponic   acid)   [29]   moieties   for   trace   elements   collection,

N-methyl-D-glucamine moiety for B collection [30], 3,4-diaminobenzoic acid moiety for As

and Se collection [31], and ethylenediamine moiety for Ag collection [32].

In this work, cross-linked chitosan (CCTS) was chemically functionalized with serine

diacetic acid moiety through the extension arm of chloromethyloxirane. The synthesized resin

was   then   packed   in   a   mini-column,   and   the   mini-column   was   installed   in

laboratory-assembled auto-pretreatment system (Auto-Pret AES System) coupled with

inductively   coupled   plasma-atomic   emission   spectrometer   (ICP-AES)   for   the

collection/concentration and determination of 13 trace elements. Experimental variables of the

system and pretreatment procedure, which are related to the improvement of sensitivity and

reproducibility, have been optimized. The proposed method provides effective automated

on-line preconcentration with high sensitivity (ppt level for several analytes), good

reproducibility, less reagent consumption, less waste, and less time-consuming.

2 Experimental

2.1 Instruments

The adsorption behavior of cross-linked chitosan functionalized with serine diacetic acid (CCTS-SDA) resin toward various trace elements was examined by inductively coupled plasma mass spectrometer (ICP-MS) SPQ8000H System Seiko Instruments (Chiba, Japan). Infrared spectra (4000–400 cm^{-1}) were recorded on Jasco FT/IR-4100 Jasco International Co. Ltd. (Tokyo, Japan). A modular digital syringe pump Cavro (San Jose, CA, USA), a selection valve and a switching valve Hamilton (Reno, NV, USA) were used as a part of Auto-Pret AES system. This Auto-pret AES system was coupled with ICP-AES Vista-pro Seiko Instruments (Chiba, Japan). The operating condition of ICP-AES is shown in Table 1.

2.2 Reagents

Flake type chitosan with 80% deacetylation degree was purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). All other reagents used for the synthesis of CCTS-SDA resin were of analytical reagent grade.

Multi-element standard solutions were prepared from several kinds of single element standard solution for atomic absorption (1000 $\mu g\ mL^{-1}$) purchased from Wako Pure Chemicals (Osaka, Japan). Two multi-element stock solutions for ICP-MS, XSTC-13 and XSTC-1 provided by Spex CertiPrep Inc. (Metuchen, NJ, USA) were mixed with the single element solutions to give a multi-element standard solution containing 63 elements. The

XSTC-13 contains 10 µg mL^{-1} of 31 elements, namely Th, Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co,

Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, TL, V, Zn, U, and Hg, and

XSTC-1 contains 10 µg mL^{-1} of 16 elements, namely Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr,

Sm, Sc, Tb, Tm, Yb, and Y.   The mixed solution was diluted with 1 M nitric acid before

column pretreatment to give a solution containing 10 ng mL^{-1} of each element. Accurate

dilution of the standard solution was carried out by weight.

Ultrapure grade nitric acid (60%, density 1.38 g mL^{-1}) Kanto Chemicals (Tokyo,

Japan) was diluted with ultrapure water to give 1 M and 2 M nitric acid. Acetic acid

(minimum 96%) and ammonia water (29%), which were used for preparing ammonium

acetate buffer solutions, were electronic industrial reagent grade Kanto Chemicals (Tokyo,

Japan). Ultrapure water (18.3 MΩ cm^{-1} resistivity) prepared by Elix 3/Milli-Q Element

system Nihon Millipore (Tokyo, Japan) was used throughout.

2.3 Synthesis of CCTS-SDA

The CCTS-SDA was synthesized in three major steps. First, in order to improve the

mechanical and chemical durability of chitosan, a cross-link structure of chitosan was made

with ethyleneglycoldiglycidylether (EGDE) as the cross-linker. The procedures are as follow

[21]: chitosan with 100-300 µm of diameter was prepared by mechanical grinding and sieving.

Smaller-size chitosan provides higher surface area, and thus can improve the adsorption

performance. However, in practical use, small size chitosan particle may reduce the flow rate of solutions in the column since internal pressure will become higher. Among two reactive groups in chitosan, hydroxyl and amino group, the amino group was preserved for further derivatization due to its reactivity, and thus the hydroxyl group was used as terminal for cross-link structure. Before cross-link structure was made, chitosan was reacted with benzaldehyde (80 g) in order to protect the amino group. The protected-chitosan was then filtered by using filter paper, and washed with ethanol to remove the remaining benzaldehyde, followed by washing with water. The cross-link structure was made by refluxing the protected-chitosan with EGDE (30 g). The product was then filtered and washed with ethanol and water, respectively. The Schiff base was cleaved to recover amino group by stirring the product in 0.5 M hydrochloric acid (500 mL). The cross-linked chitosan was then filtered and washed three times with water.

In the second step, the cross-linked chitosan was modified by introducing serine moiety through chloromethyloxirane extension arm . The cross-linked chitosan (5 g) was reacted with chloromethyloxirane (10 g) in order to attach the extension arm. The product was then filtered using filter paper, and washed each three times with ethanol and water to remove the remaining chloromethyloxirane. In separated beaker, serine (10 g) was reacted with benzaldehyde (30 g) to protect its amino group. The cross-linked chitosan, which has chloromethyloxirane as an extension arm, was then coupled with the protected-serine. The

product was then filtered and washed with ethanol, followed by water. The amino group was

recovered by stirring the product in 1 M HCl (500 mL), followed by filtration. The product

was washed with ethanol, followed by water.

In the final step, the cross-linked chitosan with serine moiety was reacted with

bromo-acetic acid solution (20 g) to convert serine moiety into serine diacetic acid moiety.

The product was then filtered and washed three times with water. The synthesis scheme of

CCTS-SDA is shown in Fig. 1.

2.4 Batch wise mini-column procedure for metal collection

One milliliter (wet volume) of CCTS-SDA resin was packed in a small polypropylene

column (5.0 mm i.d. × 50 mm) Muromachi Chemical (Kyoto, Japan). The resin was washed

by passing 10 mL of HNO_3 solution through the column, followed by passing 10 mL of

ultrapure water. The resin was then conditioned to appropriate pH by using 5 mL of

ammonium acetate solution. Sample solution (10 mL), whose pH was adjusted with the same

buffer solution for column conditioning, was passed through the column. Then, 5 mL aliquot

of 0.2 M ammonium acetate solution was passed through the column to remove un-adsorbed

metal ions, mainly hard cations such as alkaline and alkaline earth metals. The remaining

buffer solution in the column was washed out by passing 5 mL of ultrapure water. Finally, 10

mL of 1 M HNO_3 was passed through the column for recovering metal ions which were

adsorbed on the resin. The effluents were collected, and analyzed by ICP MS.

2.5 On-line metal collection and determination procedure

The manifold of Auto-Pret AES system is shown in Fig. 2. The system was composed

of a syringe pump (SP) with volume capacity of 2.5 mL, a six-port selection valve (SLV), and

a six-way switching valve (SWV). CCTS-SDA resin was packed in polytetrafluoroethylene

(PTFE) column (2 mm i.d x 4 cm), and the column was installed on SWV in the position as

shown in Fig. 2. PTFE tubing with 0.8 mm of inner diameter was used for all connecting lines,

except for holding coil where PTFE tubing with 2 mm of inner diameter was used. The

on-line pretreatment procedure was carried out in four major steps: *column conditioning*,

preconcentration, *washing*, and *elution*.

In the first step, namely *column conditioning step*, 1 mL of 0.5 M ammonium-acetate

solution (pH 7) was aspirated into holding coil at flow rate of 100 μL s^{-1} through port 3 of

SLV. While SWV was kept in load-position, the solution in holding coil was injected at flow

rate of 30 μL s^{-1} into the column in order to adjust the pH of the resin.

In the *preconcentration step*, 2.5 mL of sample was aspirated into holding coil at flow

rate of 100 μL s^{-1} through port 2 of SLV. The aspirated sample was then injected into the

column at flow rate of 20 μL s^{-1} for the adsorption of target elements on the resin as well as

the removal of matrices. This process was repeated two times to have 5 mL of sample passed

through the column.

The *washing step* was carried out by aspirating 1 mL of ultrapure water into syringe pump at flow rate of 100 µL s^{-1}, and then the aspirated-water was injected into the column at flow rate of 30 µL s^{-1}. This process will force the sample which remained in the connecting-line to pass through the column, and at the same time removes the remaining matrices and un-adsorbed metal ions.

Finally, in the *elution step*, 10 µL of air was aspirated at flow rate of 10 µL s^{-1} from the port 6 of SLV, followed by aspiration of 0.5 mL of 2 M nitric acid into holding coil at flow rate of 100 µL s^{-1} from the port 5 of SLV, and then 10 µL of air was aspirated again at flow rate of 10 µL s^{-1} from the port 6 of SLV, hence the eluent was sandwiched with air bubbles. The position of syringe pump was set to 2.5 mL by aspirating ultrapure water into the syringe pump at flow rate of 100 µL s^{-1}. This step was made to ensure that the next injection will completely transport the eluent through the column and reach ICP-AES nebulizer. After the eluent preparation was done, the position of SWV was turned to inject-position, and the eluent was injected into the column at flow rate of 30 µL s^{-1}, and the effluent zone moved to the ICP-AES for measurement.

The entire sequence described above was automatically controlled with home-made software developed by the authors, using Microsoft Visual Basic 6.

2.6 Samples

The certified reference material of river water, SLRS-4, was purchased from Chemical Metrology, Institute for National Measurement Standards, National Research Council of Canada (Ottawa, Ontario, Canada).

River water samples were collected from Zasu and Asahi River which flow through Okayama City. These samples were acidified to about pH 1 by adding concentrated nitric acid, followed by filtration with 0.45 μm membrane filter before storage.

3 Results and Discussion

3.1 Characteristics of CCTS-SDA resin

The products from each synthesis step were characterized by measuring their IR spectra. The result is shown in Fig. 3. The peak at 894 cm^{-1} in the spectrum of cross-linked chitosan confirms N–H wagging of the amino group. This peak intensity was decreased when chloromethyloxirane was attached to chitosan through the amino group. The appearance of C-Cl bonding in CCTS-chloromethyloxirane, however, is not reliable to be distinquished from CCTS since C-Cl adsorption peak falls in the region of C-H and C-C adsorption peak. The alteration of CCTS-chloromethyloxirane to CCTS-serine was marked by the appearance of sharp peak in 1743 cm^{-1}, which represents the carbonyl group from serine moiety. Another sharp peak in 1560 cm^{-1} confirms the existence of N-H scissoring of primary amine in serine

moiety. The reaction between CCTS-serine with bromo acetic acid caused the amino group in serine moiety altered to tertiary amine. This reaction was confirmed by the disappearance of peak in 1560 cm^{-1} which correspond to N-H scissoring of the primary amine.

3.2 Adsorption of various elements on CCTS-SDA

The result of adsorption behavior study is summarized in Fig. 4, expressed as recovery of each 10 μg L^{-1} of 57 elements at pH 1 to 9 using batch-wise mini-column procedure. The synthesized resin shows good adsorption toward Cd (pH 6-9), Pb (pH 6-9), Cu (pH 2-9), Ni (pH 3-9), V (pH 6-9), Ga (pH 2-9), Sc (pH 6-9), In (pH 2-9), and Th (pH 3-9). Rare earth elements (REE), sharing their similar properties, were adsorbed on the resin at neutral pH region. Almost all of the adsorbed metal ions can be quantitatively recovered with 1 M HNO$_3$.

It was reported that cross-linked structure of chitosan may hinder metal ions from accessing functional groups which bound directly to the chitosan, and therefore adsorption capability of cross-link chitosan may possibly inferior than chitosan itself [20]. However, in the present study, the serine diacetic acid functional group was introduced to the chitosan through the extension arm of chloromethyloxirane, thus providing better access for metal ions to the functional group. The proposed CCTS-SDA resin possesses three acetic acid groups which bound to the terminal of the moiety, and thus enhance chitosan capability in chelating. Additionally, a combination between chelating and ion exchange mechanism was expected to

be active since vanadium as an oxoanion can be adsorbed on the resin through ion exchange mechanism, while other elements were adsorbed through chelating mechanism. Several chelating groups which were attached on the adjacent chitosan-monomer may also coordinate metal ions. The proposed structure of metal chelation by CCTS-SDA is shown in Fig. 5:

3.3 Optimization of the auto-pretreatment system

3.3.1 Column dimension

The synthesized resin was packed in a PTFE column (2 mm i.d. x 4 cm) and the column was installed in the Auto-Pret System. The utilization of line-filter (6 mm i.d. x 4 mm packing length) as an alternative for resin packing was also studied. The result showed that the longer PTFE column is preferred, since it gave higher sensitivity, rather than the short line-filter with the nearly same amounts of packed-resin. Although it might be a good idea to keep extending the length of column, the increase of column length will lead to an increase of internal pressure inside the column, which will become one of serious drawbacks in the system.

3.3.2 Effect of sample pH and sample flow rate

In solid phase extraction studies which involve chelation, influence of aqueous solution pH is one of the important factors for quantitative collection/concentration and

recoveries of target analytes. The influence of pH in the collection and recovery of 57 elements was investigated in the ranges of 1 – 9 as described in section 3.2: the results are shown in Fig. 4. For further experiment, pH 7 was selected since it allows simultaneous determination of 13 trace elements, Cd, Pb, V, Cu, Ni, Sc, Dy, Er, Eu, Lu, Yb, Gd, and Ho. Among these metal ions, Cd, Pb, V, and Cu are toxic heavy metal ions and requisite for metal analysis in environmental water samples, and lanthanoid metals are interesting in the field of earth science.

Sample flow rate determines the contact duration between sample solution and resin in the column. If the resin adsorption rate toward analytes is fast enough, then the required contact time to achieve good adsorption efficiency will be lesser. In general, longer contact time will lead to better adsorption efficiency of the analytes on the resin. However, a compromise between sample flow rate and sample throughput should be considered. In the present study, the effect of sample flow rate on the collection of metals was examined in the ranges of 15 – 30 μL s^{-1}. Sample flow rate greater than 20 μL s^{-1} was found to cause lower peak height of the observed signal, if compared to those obtained by using flow rate lower or equal to 20 μL s-^{1}. Practically, it is preferred to have faster analysis time, thus sample flow rate of 20 μL s^{-1} was selected for further study.

3.3.3 Effect of concentration, volume, and flow rates of eluent

Nitric acid was used as the eluent in this study, and its concentration and flow rates were optimized to improve the sensitivity.

The concentration of nitric acid affects the effectiveness of desorption process, and also affects the desorption rate of adsorbed elements. Since in an on-line preconcentration system the desorbed-analytes is concentrated in a zone of eluent, rapid elution would allow analytes to be desorbed in a narrow eluent zone, and thus improve the sensitivity of the system. In contrast, less rapid elution will cause the analytes to be distributed in a wider eluent zone, and consequently decreasing the sensitivity of the system. The effect of nitric acid concentration was studied in the range of 0.5 M to 3 M. It was found that 0.5 M was not effective to elute the adsorbed elements and gave the lowest sensitivity among the concentrations studied. Quantitative recovery of the adsorbed elements can be carried out by using 1 M of nitric acid. However, related to the elution rate which affects the distribution of desorbed elements in the eluent zone, 2 M nitric acid was found to give better sensitivity than 1 M of nitric acid. Further increase in nitric acid concentration, such as 3 M of nitric acid, was found to give insignificant improvement in sensitivity compared to 2 M of nitric acid. Therefore, 2 M nitric acid was selected for further experiments.

The volume of the eluent (2 M HNO_3) was examined by varying its value from 0.25 to 0.75 mL to ensure complete elution of the analytes and provide the highest sensitivity. It was found that the peak height of obtained signals increased with the increase of eluent volume,

up to 0.5 mL. The elution with more than 0.5 mL of 2 M nitric acid, however, showed a

decreasing trend in peak height. This is because an internal dispersion occurred in the eluent

zone, as the eluent zone became longer with the increase of eluent volume. Therefore it is

preferred to trap the eluent in a shorter zone. As a compromise, 0.5 mL was selected as the

optimal volume.

The flow rate of eluent determines the contact time between eluent and resin, and

longer contact time is supposed to give a better desorption efficiency. The effect of eluent

flow rate (2 M HNO_3) was examined by varying eluent flow rate from 12 to 45 $\mu L\ s^{-1}$. It was

found that flow rate lower than 20 $\mu L\ s^{-1}$ resulted in lower peak height, due to dispersion of

desorbed-analytes in the eluent zone during its flow to ICP-AES, and flow rate larger than 30

$\mu L\ s^{-1}$ caused decreasing in peak height because of insufficient contact time. Considering the

analysis time, the eluent flow rate of 30 $\mu L\ s^{-1}$ was selected as the optimal condition for eluent

flow rate.

The effect of reversion eluent flow, in which the direction of eluent flow is opposite to

the direction of sample flow, was also examined to improve the sensitivity of the system. The

application of reversion eluent flow rate in Auto-pret system was found to give higher peak

height, compared to the result obtained when the elution direction was the same with the

sample flow. Therefore, reversion eluent flow was carried out in this work, by attaching the

mini-column on port 2 and 6 of SWV, as shown in Fig. 2, thus the eluent flow will become

reversed when SWV is switched to inject position.

3.3.4 Effect of air bubbles for sandwiching eluent zone

Air bubbles was used in the elution process in order to prevent the dispersion of eluent

zone during its flow in the holding coil, through the column, and finally reach ICP-AES,

which results in higher sensitivity. In this process, the eluent zone formed by 0.5 mL of 2 M

HNO_3 was sandwiched at both ends with air bubbles and flowed through the column. A

significant improvement of peak height was observed. The peak height increase $10 - 22\%$

compared to the elution without air bubbles. The volume of air bubbles in both ends of the

eluent zone was examined by varying the volume from 8 to 30 μL: 10 μL of air bubbles was

sufficient for sandwiching the eluent zone.

The optimized parameters of Auto-Pret System were summarized in Table 2, and an

example of flow signal of Cd in ppt levels is shown in Fig. 6.

3.4 Analytical figures of merit

The performance of the proposed method was evaluated by examining sensitivity

enhancement factor and detection limits. Linear equations from the calibration graph of trace

elements, which were mainly constructed in sub-ppb levels to ppt level, showed good

correlation coefficient for the examined elements. These values are summarized in Table 3.

The sensitivity enhancement factor was calculated from the ratio of peak height obtained by using Auto-pret system under the optimal condition, to those obtained by directly injecting the samples into ICP-AES. The sensitivity enhancement factors can vary due to the difference in the desorption rate of each element. An element which can be desorbed in higher desorption rate will be concentrated in a narrower eluent zone, and thus give a higher sensitivity enhancement factor.

Limits of detection (LOD), for each examined analytes, is defined as the concentration corresponding to the three times of signal-to-noise ratio. These results are also summarized in Table 3: LODs are varied from 0.002 μg L^{-1} (Cd) to 0.095 μg L^{-1} (Ni). Lower LOD value can be expected by increasing sample volume. From these results, the proposed method provides a significant improvement in sensitivity and enables the measurement of heavy metal ions in sub-ppb and ppt level.

3.5 Application to real river water samples

The applicability of the proposed method was verified by determining a certified reference material of river water, SLRS-4. The obtained result demonstrates that the concentration of trace elements obtained by using the proposed method is in good agreement with the certified values. Moreover, this result suggests that common matrices in river water do not interfere with the determination. Further, the method was applied to the determination

of trace elements in water samples taken from Asahi River and Zasu River, which flow through Okayama city, Japan. The result of trace elements determination by the proposed method, using 5 ml of river water sample, is shown in Table 4. The concentrations of metal ions were found to be higher than the LOD of measurement shown in Table 3, and good reproducibility was obtained, except for Cu. This is because copper cone was used in ICP-AES in this study, thus the reproducibility and LOD of Cu were inferior compared to the result of other metal ions.

4 Conclusion

A novel chitosan resin functionalized with serine diacetic acid moiety was synthesized and examined. The synthesized resin showed good adsorption behavior toward 13 trace elements: Cd, Pb, V, Cu, Ni, Sc, Dy, Er, Eu, Lu, Yb, Gd, and Ho. It was found that common matrices which exist in environmental water samples do not interfere with the determination.

The utilization of auto-pretreatment system, combined with the synthesized resin, provides fast analysis, good sensitivity, and excellent detection limit. The method was successfully applied to the determination of trace elements in river water samples, verified with a certified reference material.

5 Acknowledgement

6 References

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Figure captions

Fig. 1 Synthesis scheme of CCTS-SDA. (A) Synthesis of CCTS (B) Protection of amino group in serine (C) Synthesis of CCTS-SDA.

CCTS: Crosslinked chitosan

CCTS-SDA: Crosslinked chitosan functionalized with serine diacetic acid moiety

Fig. 2 Laboratory-assembled auto-pretreatment system manifold for on-line preconcentration and determination of trace elements.

SP: Syringe pump; V1: Syringe valve; SLV: Six-port selection valve; SWV: Six-way switching valve; MC: Mini column;

Fig. 3 FT-IR spectra; (A) CCTS (B) CCTS-chloromethyloxirane (C) CCTS-serine (D) CCTS-SDA.

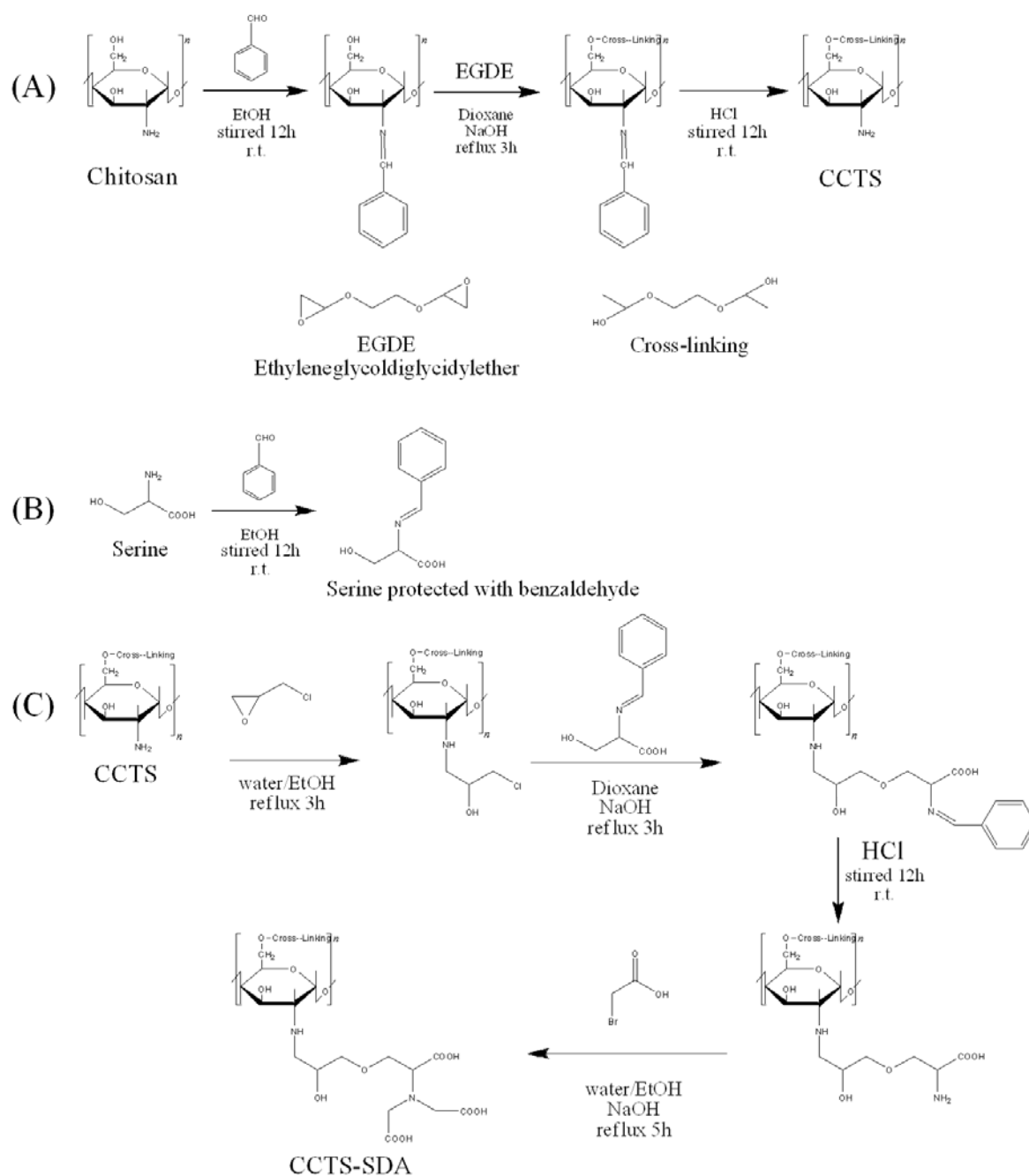Fig. 4 Adsorption behavior CCTS-SDA resin toward various elements.

Samples: 10 mL of solution containing 10 μg L^{-1} various elements; eluent: 10 mL of 1 M HNO$_3$

Fig. 5 Proposed structure of metal chelation on serine diacetic acid moiety.

Fig. 6 Example of flow signals of Cd at ppt level.

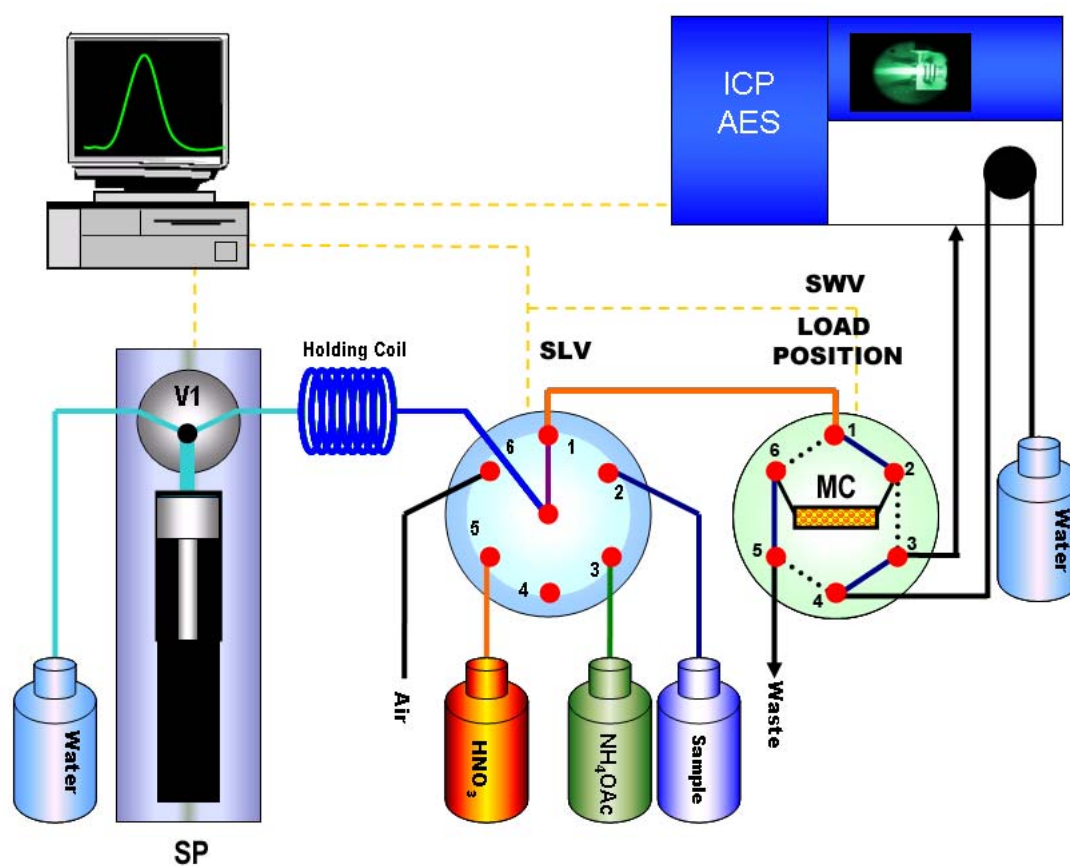Sample volume: 5 mL; other conditions are the same as summarized in Table 2.

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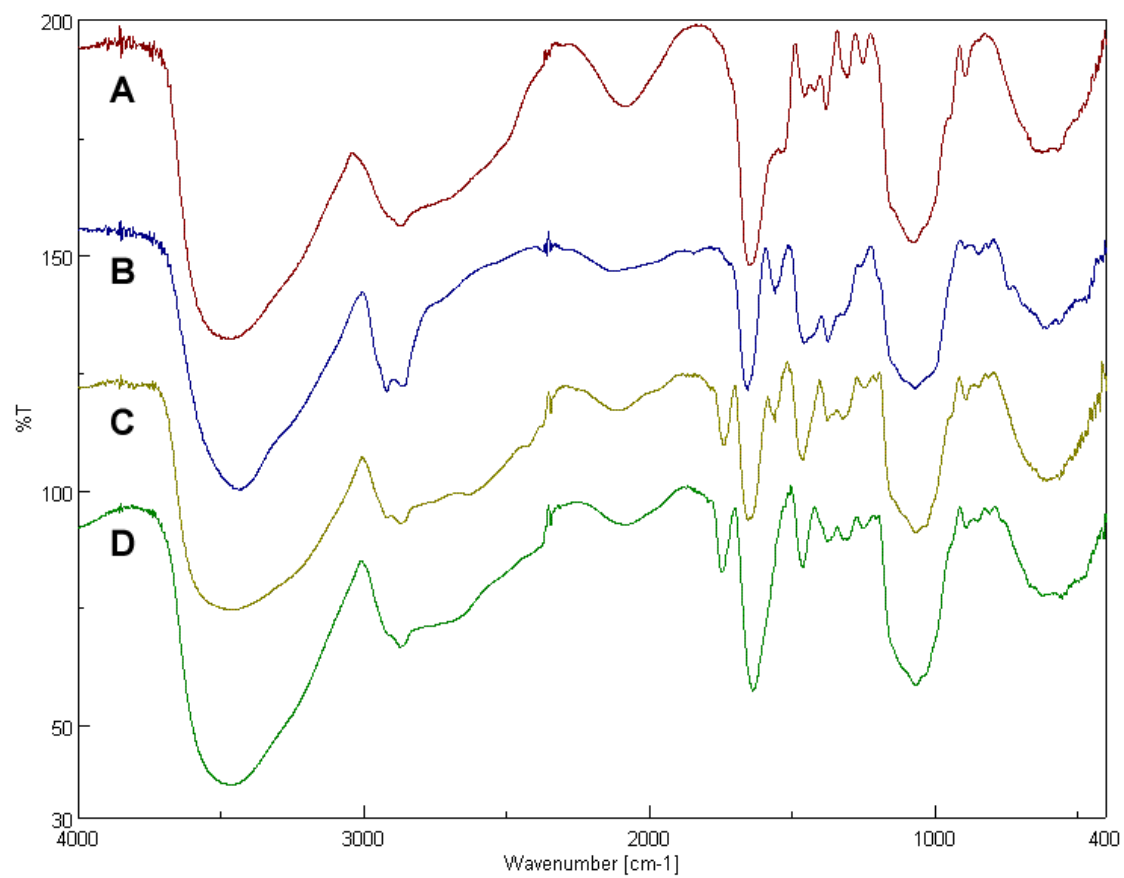
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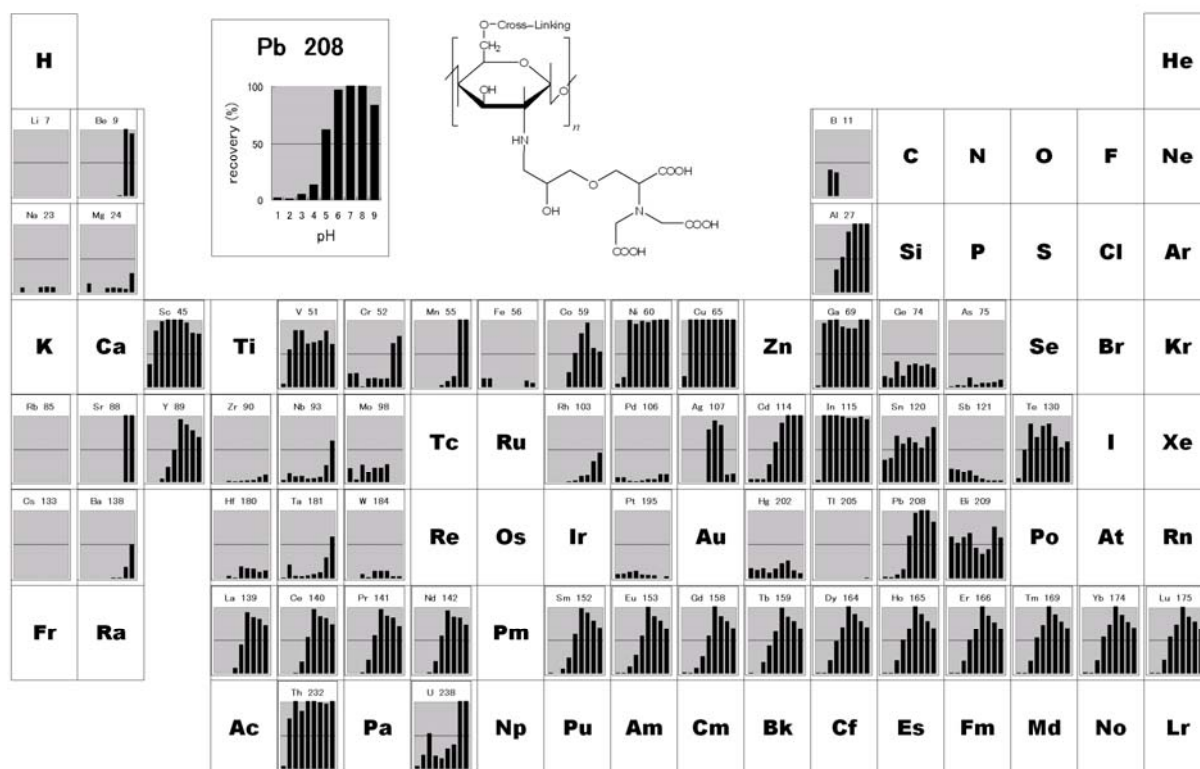
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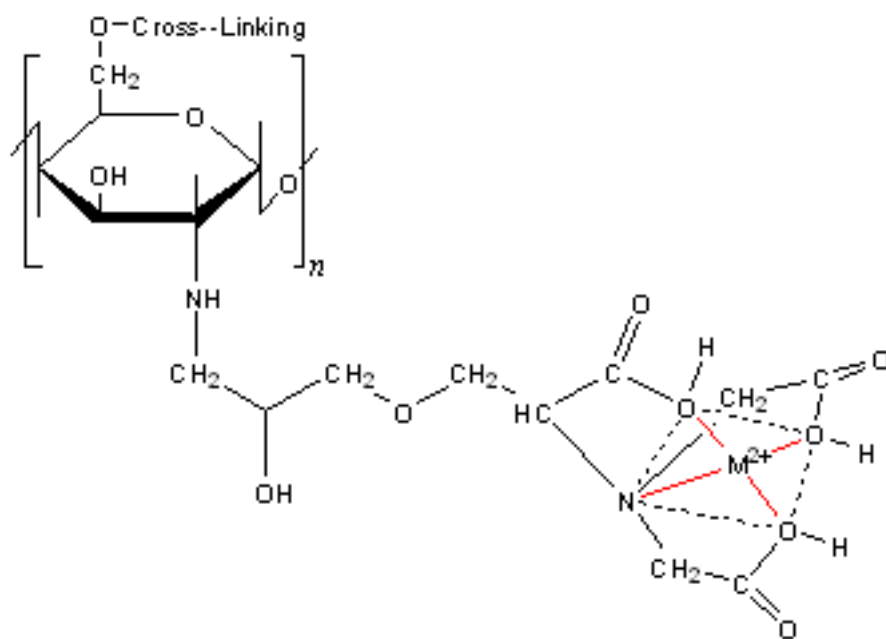
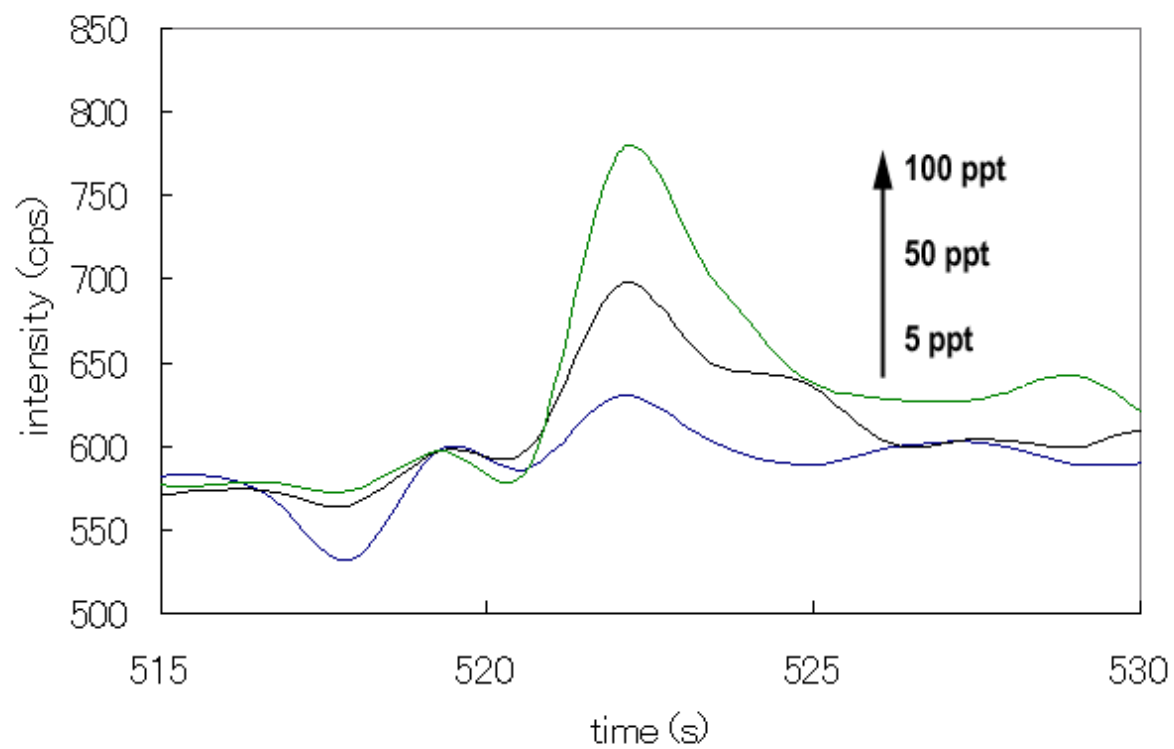**Fig. 3** *FT-IR spectra; (A) CCTS (B) CCTS-chloromethyloxirane (C) CCTS-serine (D) CCTS-SDA.*

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List of Tables

Table 1 Operating conditions of ICP-AES

Spectrometer	VISTA PRO
Plasma conditions	
RF power	40 MHz , 1.2 kW
Plasma gas flow rate (L min^{-1})	Ar 15.0
Auxiliary gas flow rate (L min^{-1})	Ar 1.50
Nebulizer gas flow rate (L min^{-1})	Ar 0.75
Spray chamber	Glass cyclonic spray chamber
Nebulizer	K-style concentric glass nebulizer
Torch	One-piece low flow extended torch in the axial view mode
Data acquision	
Measurement mode	Time scan mode

Table 2 Optimized conditions for auto-pretreatment system

Parameter	Range examined	Selected conditions
pH of sample	1-9	7
Sample loading flow rate	15-30 μL s^{-1}	20 μL s^{-1}
Eluent concentration	0.5 – 3 M HNO$_3$	2 M
Eluent volume (2M HNO$_3$)	0.25 – 0.75 mL	0.5 mL
Eluent flow rate (2M HNO$_3$)	12 – 45 μL s^{-1}	30 μL s^{-1}
Volume of air bubbles	0 – 30 μL	10 μL

Table 3 Analytical figures of the proposed method with the auto-pretreatment system

Elements	λ (nm)	R^2 [a]	SEF [b]	LOD [c] ($\mu g\ L^{-1}$)
Cd	214.349	0.999	62	0.002
Cu	324.754	0.998	61	0.024
Ni	216.555	0.990	23	0.095
V	292.401	0.991	25	0.033
Pb	220.353	0.998	33	0.036
Sc	361.383	0.994	74	0.005
Dy	353.171	1.000	84	0.039
Er	349.910	0.991	82	0.018
Eu	420.504	0.999	120	0.026
Lu	261.541	0.999	85	0.003
Yb	328.937	0.995	79	0.005
Gd	342.246	0.993	73	0.028
Ho	345.600	0.996	76	0.019

[a] Linearity of the calibration graph

[b] Sensitivity enhancement factor

[c] Correspond to the three times of signal-to-noise ratio (S/N = 3)

Table 4 Analytical results of river water samples and standard reference material

Element	Found			Certified value
	River water A[*1] (μg L^{-1})	River water B[*2] (μg L^{-1})	SLRS 4[*3] (μg L^{-1})	of SLRS-4 (μg L^{-1})
Cd	0.006 ± 0.001	0.003 ± 0.001	0.011 ± 0.001	0.012 ± 0.002
Pb	0.674 ± 0.017	0.441 ± 0.029	0.082 ± 0.004	0.086 ± 0.007
V	0.416 ± 0.014	0.490 ± 0.046	0.350 ± 0.011	0.32 ± 0.03
Cu	0.487 ± 0.026	0.861 ± 0.063	1.67 ± 0.20	1.81 ± 0.08
Ni	0.487 ± 0.017	0.486 ± 0.030	0.647 ± 0.060	0.67 ± 0.08
Sc	0.008 ± 0.0001	0.015 ± 0.0005	0.052 ± 0.008	
Dy	0.072 ± 0.001	0.081 ± 0.002	0.160 ± 0.001	
Er	0.120 ± 0.002	0.170 ± 0.030	0.100 ± 0.010	
Eu	0.066 ± 0.003	0.053 ± 0.003	0.050 ± 0.007	
Lu	0.014 ± 0.0002	0.014 ± 0.0004	0.019 ± 0.001	
Yb	0.014 ± 0.0002	0.019 ± 0.0005	0.024 ± 0.002	
Gd	0.075 ± 0.005	0.092 ± 0.008	0.119 ± 0.0008	
Ho	0.039 ± 0.001	0.031 ± 0.006	0.064 ± 0.004	

[*1] River water A was sampled at Asahi River, Okayama City, Japan;

[*2] River water B was sampled at Zasu River, Okayama City, Japan;

[*3] Standard reference material of river water;

Five milliliters of the sample was used, and experimental conditions were the same as in Table 2