

Chemistry

Analytical Chemistry fields

Okayama University

Year 2007

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Synthesis of Cross-linked Chitosan Functionalized with Threonine Moiety and Its Application to On-line Collection/Concentration and Determination of Mo, V, and Cu

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Abstract

A novel chitosan-based resin functionalized with threonine moiety was synthesized, and applied to the collection/concentration of Mo, V, and Cu in environmental water samples, followed by their determination using inductively coupled plasma-atomic emission spectrometer (ICP-AES). The synthesized resin, crosslinked chitosan-threonine (CCTS-Thr), showed good adsorption behavior toward trace amounts of Mo, V, and Cu in a wide pH range. The adsorbed elements can be easily eluted using 2 mol L⁻¹ of nitric acid, and their recoveries were found to be 90-100%. The CCTS-Thr 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 by ICP-AES. Experimental parameters related to the improvement of sensitivity and reproducibility were optimized. The limits of detection (LODs) for target metals were found to be in sub-ppb level. The proposed method with

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CCTS-Thr resin was successfully applied to the determination of Mo, V, and Cu in environmental water samples. The recovery test showed that common matrices which exist in environmental water samples did not interfere with the determination.

Keywords: Resin; Chitosan; Threonine moiety; Molybdenum; Vanadium; Copper; On-line Pretreatment; Computer control; Inductively coupled plasma-atomic emission spectrometry;

1 Introduction

Molybdenum has been recognized as an essential trace element for many species including human. It is a component of many enzymes and also participates in a large number of enzymatic reactions. A deficiency of molybdenum in human diet adversely affects growth and may lead to neurological disorders and even death [1]. On the other hand, the intake of high concentration of Mo results in severe gastrointestinal irritation and death due to cardiac failure [2]. Its excess may also cause bone deformation, teeth vulnerable to caries and disturbances in the metabolism of fats and proteins. Similar to molybdenum, vanadium is also a metal of interest in medical field due to its importance as an essential trace element which possesses specific physiological function. Both elements share left / right-sided cell receptors and are considered to be essential for human health. However, vanadium also has been recognized as a potentially dangerous pollutant in the class of lead, cadmium, and mercury [3].

Although both molybdenum and vanadium naturally exists as trace elements in earth crust, human activities intense the discharge of both metals to environmental. World production of molybdenum ores is more than 100,000 ton per year, and the world wide usage of Mo in many industry sector increases the chance of molybdenum intrusion into aquatic system and water supply. Oil and coal combustion for energy production increase the content of vanadium in environment [4]. Due to their dual effect toward living organism, both metals determination is receiving attention in pollution and nutritional studies.

The sensitivity of inductively coupled plasma atomic emission spectrometer (ICP-AES) enables the measurement of molybdenum and vanadium in ppb level, but separation techniques are often necessities prior to measurements because of serious interferences from matrix condition of samples [2, 5]. Moreover, the concentration of molybdenum and vanadium in relative clean water falls in sub-ppb level thus caused the direct measurement even more difficult to be done and adequate preconcentration step become necessary prior to measurement.

In recent years, the importance of separation and concentration techniques involving solid phase extraction in trace analysis has risen substantially. The pretreatment of aqueous solutions by the sorption technique not only can increase analyte concentrations to detectable levels, but also can eliminate matrix effects [6]. Several preconcentration methods of Mo and V, separately or simultaneously, based on solid-phase extraction have been reported [3-5,

7-17]. The utilization of commercially available amberlite XAD-7 resin [7] and polyurethane foam [11], however, required reduction of molybdenum and complexation with thiocyanate to be included before column pretreatment. Such method may raise disposal problem because organic waste will be generated continuously. Quercetin immobilized on silica gel was used to
65 selectively preconcentrate Mo [10], but the column capacity toward Mo was found to be low. Resin suspension-injection method in which cellulose nitrate resin was used to preconcentrate Mo provide low detection limit [12], but the method required tedious sample pretreatment before the column-adsorption can be carried out, and it also suggested the continuous replacement of the resin since the resin was consumed during each measurement.

70 Although the sample pretreatment with solid phase extraction method has a potential to improve the sensitivity of trace metals analysis, the operation of the pretreatment procedures may become tedious and time consuming if it is carried out in a manual batch-wise procedure where stringent control of the laboratory environment is required to avoid sample contamination, especially if the determination of trace levels of analytes is attempted. Such
75 drawback can be overcome by utilizing an automated on-line preconcentration procedure, where less contamination and less reagent consumption can be achieved [3, 18-22].

Chitosan, an N-deacetylated product of chitin which can be obtained in large quantity from crustacean shells, is a promising natural polymer for resin base material because of their unique properties, such as high chelating ability compared to other natural polymers obtained

80 from seafood wastes, easy derivatization, and biodegradability [23]. The reactive amino groups in chitosan provide easiness in introducing various moieties for wider applications in solid phase extraction. Compared to other synthetic polymers, chitosan possesses higher hydrophilicity which provides faster adsorption rate in aqueous medium.

Several researchers have reported the synthesis of cross-linked chitosans to improve the
85 chemical durability of chitosan against acid mediums along with their application [24-28]. Cross-linked chitosans itself have been derivatized with several functional groups and applied to solid phase extraction techniques. Those are serine [29] and 3,4-dihydroxybenzoic acid [30] moieties for U collection, leucine moiety for Mo collection [31], dithiocarbamate [32] and iminodi(methylphosponic acid) [33] moieties for the collection of trace elements,
90 N-methyl-D-glucamine moiety for B collection [34], 3,4-diaminobenzoic acid moiety for the collection of As and Se [35], and ethylenediamine moiety for Ag collection [36].

In this work, cross-linked chitosan (CCTS) was chemically modified with threonine moiety through the extension arm of chloromethyloxirane. The synthesized resin was packed in a mini-column, and the mini-column was installed on a laboratory assembled automated
95 pretreatment system coupled with ICP-AES (Auto-Pret AES System). The system was then utilized to carry out automated procedures for the collection and determination of Mo, V, and Cu. Experimental variables, which are related to the improvement of sensitivity and reproducibility, also have been optimized. The proposed method provides effective automated

on-line preconcentration and determination with numerous advantages such as high sensitivity,
100 good reproducibility, fast analysis, less reagent consumption, and less waste.

2 Experimental

2.1 Instruments

The adsorption behavior of 55 elements on CCTS-Thr resin was examined using
Inductively Coupled Plasma Mass-Spectrometer (ICP-MS) SPQ8000H System of Seiko
105 Instruments (Chiba, Japan). Infrared spectra ($4000\text{--}400\text{ cm}^{-1}$) of the synthesized resin were
recorded by using Jasco FT/IR-4100 Jasco International Co. Ltd. (Tokyo, Japan). A modular
digital syringe pump Cavro (San Jose, CA, USA) with volume capacity of 10 mL, a six-port
selection valve, and a six-port switching valve of Hamilton (Reno, NV, USA) were used as a
part of Automated Pretreatment System. This system was coupled with ICP-AES Vista-pro
110 Seiko Instruments (Chiba, Japan) to measure the concentration of target analytes. The
operating condition for ICP-AES is shown in Table 1.

2.2 Reagents

Chitosan, flake type with 80% deacetylation degree, was purchased from Tokyo Kasei
Co. Ltd. (Tokyo, Japan). DL-Threonine (min. 95%) was purchased from Wako Pure Chemical
115 (Osaka, Japan). All other reagents used for the synthesis of CCTS-Thr resin were of analytical
reagent grade.

Multi-element standard solutions were prepared from several kinds of single-element standard solution for atomic absorption ($1000 \mu\text{g mL}^{-1}$) 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 $10 \mu\text{g mL}^{-1}$ of 31 elements: 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 the XSTC-1 contains $10 \mu\text{g mL}^{-1}$ of 16 elements: 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 solutions 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 0.5 - 3 M nitric acid. Acetic acid (minimum 96%) and ammonia water (29%), which were used for preparing ammonium acetate buffers, were electronic industrial reagent grade Kanto Chemicals (Tokyo, Japan). Ultrapure water ($18.3 \text{ M}\Omega \text{ cm}^{-1}$ resistance) prepared by Elix 3/Milli-Q Element system Nihon Millipore (Tokyo, Japan) was used for diluting standard solution.

2.3 Synthesis of CCTS-Thr

The cross-linked chitosan derivatized with threonine (CCTS-Thr) was synthesized in two major steps: synthesis of crosslinked chitosan and introduction of threonine moiety.

140 Chitosan dissolves in acidic medium which is considered to be a serious disadvantage from the view-point of metal analysis. Therefore, in order to improve the mechanical and chemical durability of chitosan, a cross-link structure of chitosan was synthesized with ethyleneglycoldiglycidylether (EGDE) as the cross-linker. Among two reactive groups in chitosan, hydroxyl group was used as a terminal for the cross-link structure, while the reactive
145 amino group was kept for further derivatization. The detail of synthesis procedure of EGDE cross-linked chitosan had been reported by other co-workers [24].

In the second step, the cross-linked chitosan (CCTS) was chemically modified by introducing threonine moiety with the aid of chloromethyloxirane extension arm. The cross-linked chitosan (5 g) was refluxed with chloromethyloxirane (10 g) in a mixture of water
150 (50 mL) and ethanol (50 mL) for 3-h to couple cross-linked chitosan with chloromethyloxirane. The product was then filtered and washed three times with ethanol followed by water to remove the remaining chloromethyloxirane. Separately, amino group in threonine was protected as a Schiff base by reacting threonine (10 g) with benzaldehyde (30 g) in ethanol (30 mL). The protected-threonine was obtained by filtration, and the product was washed with
155 ethanol. The protected-threonine was coupled with CCTS by refluxing the mixture in

dioxane (100 mL) for 3-h. The product was filtered and washed each three times with ethanol followed by water. The product was then suspended in 1 M HCl (500 mL), and the suspension was stirred at room temperature for 12 h to cleave the Schiff base and recover the amino group of threonine. The CCTS-Thr was then filtered, and washed each three times with ethanol, followed by water. Before it was packed in a column for further application, the obtained CCTS-Thr was suspended in 100 mL of 2 M HNO₃ and stirred for 6-h to remove metals which were possibly adsorbed during synthesis process. The synthesis scheme of CCTS-Thr is shown in Fig. 1.

2.4 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 10 mL, a six-port selection valve (SLV), and a six-way switching valve (SWV). CCTS-Thr resin was packed in a 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 inner diameter was used for all connecting lines, except for holding coil where PTFE tubing with 1.6 mm inner diameter was used. The on-line pretreatment procedure was carried out in four major steps: *column conditioning*, *preconcentration*, *washing*, and *elution*.

Column conditioning step: 2 mL of 0.5 M ammonium acetate buffer solution (pH 5) was aspirated at flow rate of 400 $\mu\text{L s}^{-1}$ into the holding coil through port 3 of SLV. While the SWV was kept in the load position, the solution in the holding coil was injected at flow rate of

175 $40 \mu\text{L s}^{-1}$ into the column in order to adjust the pH of the resin.

Preconcentration step: 5 mL of sample was aspirated at flow rate of $400 \mu\text{L s}^{-1}$ into the holding coil through port 2 of SLV. The aspirated sample was then injected into the column at flow rate of $30 \mu\text{L s}^{-1}$ for the adsorption of target analytes on the resin and the removal of the matrices.

180 The *washing step* was carried out by aspirating 1 mL of ultrapure water at flow rate of $400 \mu\text{L s}^{-1}$ into the syringe pump, and then the water was injected into the column at flow rate of $40 \mu\text{L s}^{-1}$. This process will force the sample remained in the line to pass through the column, and at the same time remove matrices and un-adsorbed metal ions.

Finally, in the *elution step*, 0.5 mL of 3 M nitric acid was aspirated into the holding coil
185 at flow rate of $400 \mu\text{L s}^{-1}$ from port 5 of SLV, and. The position of syringe pump was set to 2.5 mL by aspirating ultrapure water into the syringe pump. This step was made to ensure that the next injection will completely drive the eluent passing through the column and reach ICP-AES. After the eluent preparation had been done, the position of SWV was turned to inject-position, and the eluent was flowed into the column at flow rate of $25 \mu\text{L s}^{-1}$, and the effluent zone moved
190 into the ICP-AES for measurement.

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

2.5 Adsorption behavior study

The adsorption behavior of 55 elements on CCTS-Thr resin was investigated by using
195 the same procedure as for on-line metals determination described in section 2.4, with a few
exceptions as follow: the injected sample volume in *preconcentration step* was 2.5 mL, the
eluent in *elution step* was 2.5 mL of 2 M HNO₃, and the effluent was collected in
polypropylene test-tubes instead of being flowed into ICP-AES. The collected eluent was then
measured using ICP-MS. The buffer solutions used in *column conditioning step* were
200 prepared by mixing ammonium acetate (NH₄OAc) with acetic acid (HOAc), ammonia, and
nitric acid as follow: 0.5 M NH₄OAc + HOAc (pH 4 – 6), 0.5 M NH₄OAc + NH₃ (pH 7 – 9),
and 0.5 M NH₄OAc + HNO₃ (pH 1 – 3).

2.6 Adsorption capacity study

Adsorption capacity of CCTS-Thr toward Mo, V, and Cu was investigated for each
205 individual species. Stock solution of Mo, V, and Cu was prepared separately by dissolving
(NH₄)₆Mo₇O₂₄·4H₂O, NH₄VO₃, and CuSO₄·5H₂O, respectively, with 0.5 M ammonium
acetate solution. Then into each stock solution, 0.1 g (dry weight) of CCTS-Thr was added
and the mixture was stirred for 24 hours. The adsorption capacity of CCTS-Thr toward each
metal ion was calculated from the difference of initial concentration and final concentration of
210 the metal ions in the solution.

2.7 Samples

River water samples were collected from Zasu and Asahi River which flow through Okayama city, Japan. Tap water was collected from a tap water faucet located in Faculty of Science of Okayama University, in which the water was discarded 30 min before sampling.

215 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-Thr resin

The products obtained from each synthesis step were characterized by measuring their
220 IR spectra. The alteration of CCTS to CCTS-threonine was marked by the appearance of peak in 1739 cm^{-1} , which corresponds to carbonyl vibration of carboxylic acid group from threonine moiety. The intensity of this peak, however, is weaker compared to peak in 1637 cm^{-1} which represents carbonyl group from chitin monomer which may exist in chitosan as a result from incomplete deacetylation process. Peak shifting from 1526 cm^{-1} to 1559 cm^{-1} confirms the
225 difference of N-H scissoring of primary amine in chitosan and the primary amine in threonine moiety. This is also supported by the shifting of N-H wagging peak from 897 cm^{-1} to 827 cm^{-1} .

3.2 Adsorption behavior of trace elements on CCTS-Thr

The result of CCTS-Thr adsorption behavior study is summarized in Fig. 3, expressed
230 as the recovery of each 10 $\mu\text{g L}^{-1}$ of 55 elements at pH 1 to 9 using the procedure described in

section 2.5. Almost all of the adsorbed metal ions can be quantitatively recovered with 2 M HNO₃ as the eluent. The synthesized resin shows good adsorption capability toward Mo (pH 2-6), V (pH 6-9), Cu (pH 2-9), and Ga(pH 3-9). Among these metal ions, Mo, V, and Cu have a potential to harm human and environment because of their toxicity at certain concentration level.

The base material of CCTS-Thr resin, that is CCTS, has ability to adsorb Mo, V, Cu, and Ga. However, compared to its base material, the adsorption of CCTS-Thr toward Mo is superior in term of pH range, recovery value, and adsorption capacity. In the case of V and Cu, CCTS cannot quantitatively adsorb and desorb V (recovery was less than 80%), and it can only adsorb Cu at pH \geq 5 [24]. The derivatization of CCTS with threonine moiety had improved the adsorption capability toward V into wider pH range with higher recovery value. An improvement in Cu adsorption was also observed where Cu can be quantitatively adsorbed and desorbed at pH \geq 4.

3.3 CCTS-Thr adsorption capacity

It was reported that the introduction of cross-linked structure may reduce chitosan adsorption capacity toward Mo by 2 to 2.6 times, because the metals accessibility to the sorption sites changes due to the modification of polymer structure [23]. Although the reduction of chitosan adsorption capacity toward Mo also might be attributed to the saturation of amine sites for cross-link structure terminal, our observation shows that even the CCTS

250 whose EGDE cross-link terminal does not involve amine sites also has a lower adsorption capacity compared to the chitosan itself. However, in the present study, the threonine functional group was introduced to the chitosan through the extension arm of chloromethyloxirane, thus providing better access for the metal ions to the functional group. Based on the result obtained using batch-wise procedure, the adsorption capacity of

255 CCTS-Thr toward Mo was found to be 3.47 mmol g^{-1} . Compared to CCTS-leucine [31] and epichlorohydrin-CCTS [27], the adsorption capacity of CCTS-Thr toward Mo is superior because the amino group in threonine moiety, which can act as a functional group to adsorb Mo(VI), is not saturated. In CCTS-leucine, the amino group was used to couple leucine with CCTS, thus its adsorption capacity toward Mo became lower. The adsorption capacity of

260 CCTS-Thr toward V and Cu was found to be 0.92 and 0.62 mmol g^{-1} , respectively. The capacity of this resin for adsorbing Cu is superior than CCTS-leucine, and nearly equal to CCTS-serine [29].

A combination between chelating and ion-exchange mechanism was expected to be active since Mo(VI) and V(V) as their oxoanion can be adsorbed on the resin through anion

265 exchange mechanism and hydrogen bonding, while Cu(II) can be adsorbed through chelating mechanism. Several chelating groups which were attached on the adjacent chitosan-monomer may also coordinate with the Cu ions. Thermodynamic data suggest that Mo should have an oxidation state of Mo(VI) in oxygenated waters [2], and Mo principally occurs as molybdate

(MoO_4^{2-}) ions [37]. Vanadate ion at pH 4-8 exists as H_2VO_4^- species, if its concentration is lower than $10^{-3.5}$ M [38]. The adsorption mechanism of Mo(VI), V(V), and Cu(II) is shown in Fig. 4:

3.4 Optimization of the auto-pretreatment system

3.4.1 Effect of sample pH and sample flow rate

In solid phase extraction studies, the influence of pH is one of the important factors for quantitative collection and elution of the analytes. Moreover, element such as molybdenum and vanadium exists as oxoanion with several forms whose existence are depend on pH of the medium.

The influence of pH to the collection and recovery of 55 elements was investigated at the ranges of 1 – 9 as described in section 2.5 and the results are shown in Fig. 3. The resin shows good selectivity toward Mo in low pH, especially at pH 3 where only Mo can be quantitatively adsorbed. This pH can be selected to analyze the content of Mo(VI) selectively in environmental water sample. On the other hand, simultaneous adsorption of Mo(VI), V(V), and Cu(II) can be carried out in pH 5, and thus this pH can be selected if simultaneous analysis of Mo(VI), V(V), and Cu(II) contents in sample is attempted. In this work, pH 5 was selected for simultaneous enrichment of Mo(VI), V(V), and Cu(II), followed by their determination using ICP-AES.

Sample flow rate determines the contact time of sample solutions with the resin in the

column. In general, longer contact time will result in better adsorption efficiency of the analytes on the resin. However, a compromise between the sample flow rate and the sample throughput should be considered. In the present study, the effect of the sample flow rate on the collection of metals was examined in the ranges of 20 – 40 $\mu\text{L s}^{-1}$. It was observed that the variation of sample flow rates did not give significant effect to the adsorption of Mo, V, and Cu, because the adsorption rate was considerably fast due to the hydrophilicity of chitosan. However, increasing flow rate to be more than 50 $\mu\text{L s}^{-1}$ caused leaking in the system due to the pressure inside chitosan column. In this study, the flow rate of 30 $\mu\text{L s}^{-1}$ was chosen as the compromise of sensitivity and analysis time.

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

Nitric acid, whose spectrum is similar to water in ICP-AES system, was used as the eluent in this study. Its concentration and flow rates were optimized to improve the sensitivity of the system.

The concentration of nitric acid affects the effectiveness of the desorption process, and also affects the desorption rate. Since in an on-line preconcentration system the desorbed analytes are concentrated in the tip of eluent zone, rapid elution would allow analytes to be desorbed and concentrated in a narrow zone, and thus improve the sensitivity of the system. In contrast, less rapid elution would cause the analytes to be distributed in a wider zone, which leads to lower sensitivity. 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 2 M of nitric acid. However, considering the elution rate
310 which affected the distribution of the desorbed analytes in the eluent zone, 3 M nitric acid was found to give the best sensitivity, and therefore it was used for further experiments.

The effect of eluent (3 M HNO₃) volume was examined by varying its value from 0.25 to 1.5 mL to ascertain the complete elution of the analytes, and at the same time to provide the highest sensitivity. It was found that the highest peak height was obtained when 0.5 mL of
315 eluent was used, thus this volume was used in the further experiment.

The flow rate of eluent determines the contact time of the eluent with the resin, and longer contact time is supposed to give better desorption efficiency. The effect of eluent flow rate was examined by varying the flow rate between 20 and 40 $\mu\text{L s}^{-1}$. It was found that the flow rate larger than 30 $\mu\text{L s}^{-1}$ cause a significant decrease in the peak height because of
320 insufficient contact time. Based on such result, the eluent flow rate of 25 $\mu\text{L s}^{-1}$ was selected as the optimal condition.

In solid phase extraction, target analytes are trapped at the front-end of column where the sample solution began to flow in. If the elution is carried out in the same direction with sample flow, a dispersion of analytes in eluent zone may occur since the effluent will pass
325 along the column. This situation can be avoided by reversing the eluent flow, where eluent

will first pass through the column, and at the end of column the adsorbed analytes will be desorbed and directly enter the connection lines to ICP-AES. Therefore, the reversed eluent flow was used in further experiments by installing the mini column on the switching valve as shown in Fig. 2.

330 The optimized parameters of the auto-pretreatment system were summarized in Table 2, and an example of flow signal of Mo in sub-ppb level is shown in Fig. 5.

3.5 Analytical figures of merit

The performance of the proposed method was evaluated by examining the sensitivity enhancement factor and the detection limits obtained by using the Auto-Pret AES System. The correlation coefficient of the linear equations for the calibration graph of trace elements, which were constructed at sub-ppb levels, showed good linearity for the examined elements ($R^2 \geq 0.99$).

The sensitivity enhancement factors were calculated from the slope ratio of linear equation obtained under the optimal conditions by the Auto-Pret AES System to those obtained by directly injecting the samples into ICP-AES. The sensitivity enhancement factor was found to be 13, 26, and 35 for Mo, V, and Cu, respectively. Elution in an online system creates a segment of concentrated analytes in the tip of eluent zone. The concentration of the analytes in such segment is greatly affected by retention strength of resin toward the analytes. Strong retention causes adsorbed analytes cannot be instantly collected and the analytes will be

345 distributed in wider zone thus caused lower sensitivity. This explains why the sensitivity enhancement factor of Mo is inferior to V and Cu.

Limits of detection (LODs) for the examined analytes are defined as the concentration corresponding to the three times of the signal-to-noise ratio. LODs were found to be 0.09, 0.09, and 0.05 $\mu\text{g L}^{-1}$ for Mo, V, and Cu, respectively. Lower detection limits can be expected when
350 larger volume of sample is used. These values are summarized in Table 3. From these results, the proposed method provides a significant improvement in sensitivity and enables the measurement of Mo, V, and Cu to be carried out in sub ppb levels.

3.6 Application to real environmental water samples

The method was applied to the determination of Mo, V, and Cu in river water and tap
355 water samples. The results obtained by the proposed method using 5 ml of the sample solutions, are shown in Table 4. Since it was found that V(IV) is stable only at pH 2 and gradually oxidized at higher pH [39], and since all water samples used in this work were initially acidified by using concentrated HNO₃ which has property of oxidizing acid, it can be assumed that V(IV) which exists in low concentration was oxidized to V(V), and thus the
360 determination result reflected the concentration of total V. The concentration of metal ions was found to be higher than the LODs of measurement, and good reproducibility was obtained. The results from recovery tests, which were carried out by spiking a recorded-amount of analytes, indicated that the existence of matrices in environmental water samples did not interfere with

the determination.

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4 Conclusion

A novel chitosan resin functionalized with threonine moiety was synthesized and its potential to be applied in solid phase extraction method was examined. The synthesized resin showed good adsorption capability toward Mo, V, and Cu. Simultaneous adsorption of those metals can be carried out in pH 5, while selective adsorption of Mo can be carried out in pH 3.

The utilization of the synthesized resin along with the Auto-Pret AES System provides fast analysis, good sensitivity, and excellent detection limit. The method was successfully applied to the determination of Mo, V, and Cu in river water and tap water samples. The recovery test showed excellent recovery values, and indicated that common matrices which exist in environmental water samples do not interfere with the determination.

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Figure captions

Fig. 1 Synthesis scheme of (A) CCTS, (B) protected-threonine; (B) CCTS-Thr;

CCTS: Crosslinked chitosan

430 CCTS-Thr: Crosslinked chitosan functionalized with threonine moiety

Fig. 2 Laboratory-assembled automated-pretreatment system (Auto-Pret AES System)

manifold for on-line preconcentration and determination of Mo, V, and Cu.

SP: Syringe pump; V1: Syringe valve; SLV: Six-port selection valve; SWV: Six-way

435 switching valve; MC: Mini column;

Fig. 3 Adsorption behavior of CCTS-Thr resin toward various elements.

Samples: 2.5 mL of solution containing $10 \mu\text{g L}^{-1}$ various elements; eluent: 2.5 mL of 2 M

HNO_3

440

Fig. 4 Proposed adsorption mechanisms of Mo, V, and Cu adsorption on CCTS-Thr.

Fig. 5 Example of flow signals of Mo determination.

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

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