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Functionalization of chitosan with 3-nitro-4-amino benzoic acid moiety and its application to the collection/concentration of molybdenum in environmental water samples

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Abstract

A chitosan resin functionalized with 3-nitro-4-amino benzoic acid moiety (CCTS-NABA resin) was newly synthesized for the collection/concentration of trace molybdenum by using cross-linked chitosan (CCTS) as base material. The carboxyl group of the moiety was chemically attached to amino group of cross-linked chitosan through amide bond formation. The adsorption behavior of molybdenum as well as other 60 elements on the resin was examined by passing the sample solutions through a mini-column packed with the resin. After the elution of the elements collected on the resin with 1 M HNO₃, the eluates were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) and -atomic emission spectrometry (ICP-AES).

The CCTS-NABA resin can adsorb several metal ions, such as vanadium, gallium, arsenic, selenium, silver, bismuth, thorium, tungsten, tin, tellurium, copper, and molybdenum at appropriate pHs. Among these metal ions, only molybdenum could be adsorbed almost completely on the resin at acidic regions. An excellent selectivity toward molybdenum could be obtained at pH 3-4. The adsorption capacity of CCTS-NABA resin for Mo (VI) was 380 mg g⁻¹ resin. Through the column pretreatment, alkali and alkaline earth metals in river water and seawater samples were successfully removed.

The CCTS-NABA resin was applied to the adsorption/collection of Molybdenum in river water and seawater samples. The Concentrations of molybdenum in river water samples were found in the range of 0.84 and 0.95 ppb (ng g⁻¹), whereas molybdenum in seawater was about 9 ppb. The validation of the proposed method was carried out by determining molybdenum in the certified reference materials of SLRS-4, CASS-4, and NASS-5 after passing through the CCTS-NABA resin; the results showed good agreement with the certified values.

Keywords: chitosan resin, 3-nitro-4-amino benzoic acid, molybdenum, adsorption behavior, water and seawater, ICP-MS, ICP-AES, certified reference materials

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Introduction

Molybdenum is one of the biologically essential microelements for all classes of organisms, because of its important role in enzymatic processes, and stimulating the synthesis of proteins and nucleic acids. However, over intake of molybdenum will result in molybdenum poisoning [1-2]. Molybdenum is also widely used in industrial processes as substance for metal alloys, pigments, lubricant, and chemical catalysis [3], which can increase the level of molybdenum released to environment. Molybdenum in natural water exists in the form of MoO_4^{2-} (Mo(VI)) and its protonated species, whose concentrations are very low [4]. In Japan, the concentration of molybdenum in environmental waters, such as drinking, river, and seawaters, were reported to be 0.11-6.2, 1, and 10 ppb, respectively [5-6]. Accordingly, precise and accurate, as well as selective separation and sensitive analytical methods for the determination of molybdenum in environmental samples should be developed.

Direct determination of molybdenum in environmental samples by using spectroscopic analytical techniques, such as flame and graphite furnace atomic absorption spectrometry (FAAS and GFAAS), inductively coupled plasma-mass spectrometry (ICP-MS) and -atomic emission spectrometry (ICP-AES) are difficult because of its low concentrations and/ or

matrix interferences. In order to overcome these problems, the separation and preconcentration procedures are required [7-10].

Santos *et al* [11] reported the enrichment of molybdenum in seawater. The method is based on the complexation of Mo (VI) with by calmagite reagent and its sorption onto activated carbon. Molybdenum as calmagite complex could be quantitatively adsorbed (> 90%) onto activated carbon. However, the heating of loaded activated carbon with concentrated nitric acid at 120°C was required for desorption of molybdenum before its measurement by ICP-AES. The separation of molybdenum based on its chelation with quercetin immobilized on silica gel in a slightly acidic medium (pH 5) was reported by Azeredo *et al* [12]. Molybdenum could be recovered quantitatively (95-99%). Nevertheless, the use of organic eluent, α -benzoinoxime, for the desorption of molybdenum prior to its determination by ICP-AES, could not be avoided. Octadecyl silica sorbent and silica-based strongly basic anion exchanger-packed in a mini-column have been reported for the separation and preconcentration of molybdenum [13]. In this method, small amount of NO_3^- , SO_4^{2-} , PO_4^{3-} , K^+ , Na^+ , Mg^{2+} , Ca^{2+} , Al^{3+} , and Fe^{3+} did not interfere with the determination of molybdenum by ICP-AES. The complicated procedures, in which evaporation and elimination of organic solvent (96% ethanol) and excess mineral acid (HNO_3) after elution of molybdenum from mini-column, is the drawback of this method.

The use of chitosan as a solid support for an ion exchange and a chelating resin is

increasing due to its advantages, such as easy derivatization of its amino groups and more hydrophilic characteristic than such synthetic base materials as polystyrene-divinylbenzene, polyethylene and polyurethane [14-15]. These advantages provide fast reaction rate in sorption and desorption kinetics of analyte species in aqueous media. However, chitosan itself will easily dissolve in acidic solutions due to the protonation of its amino groups. Therefore, cross-linking agents such as ethyleneglycoldiglycidylether (EGDE) and glutaraldehyde [16-17] were used for improving its chemical stability and mechanical strength. In our previous work [18], the cross-linked chitosan functionalized with leucine moiety was synthesized for the collection/concentration of molybdenum. It was found that molybdenum could be adsorbed quantitatively on the resin from pH 1 to pH 5, whereas alkaline and alkaline-earth metals could not be retained on the resin. The adsorption capacity of the resin for molybdenum was about 96 mg Mo(VI) g⁻¹ resin.

In this work, a new chitosan resin for adsorption/collection of molybdenum was developed. The moiety of 3-nitro-4-amino benzoic acid (NABA) was chemically bonded to the amino group of cross-linked chitosan through the amide bond formation (CCTS-NABA resin). The adsorption capacity of CCTS-NABA resin for molybdenum was 380 mg Mo(VI) g⁻¹ resin, which is superior to the leucine-type chitosan resin [18], as well as superior to other molybdenum resins reported so far. The collected molybdenum on the CCTS-NABA resin is easily eluted with 1 M nitric acid. The resin was successfully applied to the

adsorption/collection of molybdenum in river water and seawater samples before its measurement by ICP-AES and ICP-MS. The certified reference materials of river water (SLRS-4), nearshore seawater (CASS-4), and open ocean seawater (NASS-5) were also examined. The results obtained by using the proposed method were in good agreement with the certified values. In addition, the matrices commonly existing in river and seawater samples were successfully removed by using this resin.

Experimental

Reagents and materials

Flake-type chitosan and 3-nitro-4-amino benzoic acid (NABA) were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). All other reagents used for the synthesis of CCTS-NABA resin were of analytical reagent grade.

Multielement standard solutions including molybdenum were prepared by diluting several kinds of a single element standard solution for atomic absorption spectrometry (1000 ppm) purchased from Wako Pure Chemicals (Osaka, Japan) and a multi-element standard solution for ICP-MS; XSTC-13 and XSTC-1 (10 ppm) provided by Spex CertiPrep Inc. (Metuchen, NJ, USA).

To examine the adsorption capacity of CCTS-NABA resin toward Mo (VI), ammonium heptamolybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O) from Wako pure chemicals (Osaka,

Japan) was used.

Ultrapure grade nitric acid (60 %, density 1.38 g ml⁻¹ Kanto Chemicals, Tokyo, Japan) was diluted with ultrapure water to give a 1 M and a 2 M acid solutions for column treatment. Acetic acid (minimum 96 %) and ammonia water (29 %) used for the preparation of ammonium acetate buffer solution were of an electronic industrial reagent grade (Kanto Chemicals, Tokyo, Japan).

Instrumentations

The ICP-MS, which was used for the measurement of molybdenum ($m/z = 98$), as well as other 60 elements, was a SPQ 8000H (Seiko Instruments, Chiba, Japan). An ICP-AES system (Vista Pro, Seiko Instruments, Chiba, Japan) was used for the measurement of molybdenum at wavelength of 202.032 nm. The operating conditions of ICP-MS and ICP-AES were almost similar to those reported in the previous work [15, 18]. Infrared spectra (4000-400 cm⁻¹) were taken by a KBr pellet method using a FT/IR-4100 spectrometer, JASCO Co. (Tokyo, Japan). An automatic titration system Model AT-510, Kyoto Electronics Manufacturing Co. (Kyoto, Japan), was used for the acid-base titration to estimate the pKa values of CCTS-NABA resin.

Preparation of CCTS-NABA resin

Cross-linked chitosan (CCTS) with the cross-linker of ethyleneglycoldiglycidylether

(EGDE) was synthesized in a similar manner to the previous work [15, 18-20]. Amino group of 3-nitro-4-amino benzoic acid moiety (NABA, 10 g) was protected with benzaldehyde as in the previous work [20] before its reaction with CCTS. Then, the CCTS-NABA resin was synthesized by reacting CCTS with amino-protected NABA moiety through the amide bond formation as shown in Fig. 1. In this step, 16 g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), 12 g of triethylamine (TEA) and 1 g of 1-hydroxybenzotriazole were mixed with amino-protected NABA in 60 mL of dimethyl formamide (DMF). Then, 5 g of the CCTS was suspended into the solution; the mixture was stirred for 20 hours at room temperature. The product was filtered off using a glass filter and washed with methanol and water. In the last step, the protection group (benzaldehyde), which was condensed with amino groups of NABA, was removed by stirring the product in 50 ml of 0.5M HCl for 12 h at room temperature; this procedure was repeated two times. Finally, the product (CCTS-NABA) was filtered on the glass filter and washed with ethanol and water.

Procedures for column pretreatment of sample solutions

The CCTS-NABA resin synthesized in this work was washed by stirring the resin in 2M HNO₃ for 6 h to remove residual metal impurities during the production of chitosan and the synthesis of CCTS-NABA. Then, the resin was filtered on a filter paper, and rinsed with the ultrapure water. A 1-mL portion of the resin, which corresponds to 0.2 g of dry weight, was

packed in polypropylene mini-columns (5 mm i.d. x 50 mm), Muromachi Chemical (Kyoto, Japan) for the examination of the adsorption behavior of the resin towards 61 elements.

The column pretreatment procedures are similar to our previous work [15, 18-21]. The resin, packed in the mini-columns, was washed with each 10-mL of 2 M nitric acid and ultrapure water. Then, 5-mL of buffer solution (pH 1-2: nitric acid; pH 3-9: 0.5 M ammonia-acetate solutions) was passed through the column for conditioning. Sample solutions (10 mL), whose pHs were adjusted with the same buffer as the one for the column conditioning, were passed through the column. A 5-mL aliquot of a 0.2 M buffer solution (pH 1-9) was then passed through the column to remove matrix ions remained on the resin, such as alkali and alkaline earth metals. In order to rinse the remaining buffer components in the column, 5-mL of ultrapure water was passed through the column. Finally, 10-mL aliquot of 1 M nitric acid was passed through the column to recover the elements adsorbed on the resin, and the eluate was measured by ICP-AES and ICP-MS. For the determination of molybdenum with 10-fold preconcentration, 100 mL of the sample solution was used, and was adjusted to pH 4.

Adsorption capacity

The adsorption capacity of CCTS-NABA resin for Mo (VI) was examined by equilibrating 0.2 g of CCTS-NABA resin in the presence of Mo (VI) solution (100 mL, 1000

ppm). The acidity of the solution was adjusted to pH 4. The solution was stirred, and at certain time interval, 0.1 mL of the solution was transferred to a clean bottle until equilibrium was reached. Then, the concentration of Mo (VI) ion remained in the solution was measured by ICP- AES after 100-fold dilution.

Sampling and preserving of water samples

River water samples were collected from Zasu and Asahi rivers, which flow through Okayama City. The seawater sample was taken at Shibukawa Sea located at Okayama Prefecture, Japan. The original pHs of the samples were measured; they were in the range of 6.8-7.2. These samples were acidified to about pH 1 by adding a small amount of concentrated nitric acid before filtration with 0.45 μm membrane filter. Before being flowed through the mini-column packed with CCTS-NABA resin, the samples were adjusted to pH 4. The sampling and analysis of the water samples were performed within the same day.

The certified reference materials of the river water (SLRS-4), the nearshore seawater (CASS-4) and the open ocean seawater (NASS-5), which are issued by the National Research Council Canada (NRCC), were used for the validation of the proposed method.

Results and discussion

Characteristics of CCTS-NABA resin

The IR spectrum of CCTS-NABA resin, in comparison with the one of cross-linked chitosan (CCTS), depicted several bands at 1541.81 cm^{-1} , and 1707.21 cm^{-1} , which are attributed to $-\text{NO}_2$ of NABA moiety and $\text{C}=\text{O}$ of the amide bond, respectively. These bands showed the existence of NABA moiety attached to CCTS in the synthesized resin.

The result of acid-base titration for the synthesized CCTS-NABA resin in an acidic solution with 0.1 M NaOH as a titrant was shown in Fig. 2. It can be expected from the chemical structure of CCTS-NABA resin that there must be seen one pK_a value, which comes from the amino group of NABA moiety. For estimation, the pK_a value of 2-nitroaniline, which is attributed to $-\text{NH}_2$ group ($\text{pK}_a: -0.25$) can be referred [22]. As shown in Fig.2, a pK_a value of 9.0 , can be observed clearly in the titration curve. The pK_a of 9.0 in resin seemed to be attributed to the unreacted amino group of cross-linked chitosan (CCTS). The pK_a value of amino group at *para*-position of NABA cannot be observed at the titration curve because it is extremely low. In this experiment, 2 ml of 0.10 M HCl was added to the resin-suspended solution (30 ml water) before titration. Therefore, the pH of HCl solution is about 2.2 , whereas $-\text{NH}_2$ group of NABA is seemed to be about -0.25 of pK_a , which may result in incomplete protonation of $-\text{NH}_2$ group.

As shown in the titration curve (Fig.2), the first end point (A) corresponds to the neutralization of 2 ml of 0.1 M HCl and partly protonated $-\text{NH}_2$ group of NABA moiety; that is, 2 ml of 0.1 M NaOH corresponds to 2 ml of 0.1 M HCl , and 1.2 ml of 0.1 M NaOH

corresponds to partly protonated of $-\text{NH}_2$ group of NABA moiety. The second end point (B) corresponds to the neutralization of the protonation of unreacted amino group of CCTS. A 2.0 ml of 0.1 M NaOH was required, which means that about 0.2 mmol in 0.2 g resin ($\approx 1.0 \text{ mmol g}^{-1}$) of amino group remains in the resin as $-\text{NH}_2$.

Assuming that NABA moiety is attached to all glucamine unit of chitosan, about 2.5 mmol NABA may exist in 1 g of the resin. However, from the titration result, it can be calculated that the amount of NABA moiety chemically bonded to CCTS is about 1.5 mmol g^{-1} ($2.5 - 1.0 \text{ mmol g}^{-1}$). Such result indicated that about 40% of $-\text{NH}_2$ of NABA Moiety could be protonated in the first end point of the titration (A). Accordingly, the mole ratio of NABA moiety to glucamine unit of chitosan can be estimated to be 0.6:1.0 (1.5 : 2.5); that is, 60% of amino group of CCTS is bound to NABA moiety.

The adsorption capacity of CCTS-NABA was obtained by equilibrating the resin with Mo (VI) solution at pH 4. The adsorption capacity of this resin for Mo (VI) was found to be 380 mg g^{-1} resin. The loading half time ($t_{1/2}$), defined as the time necessary for the adsorption of a half of its maximum adsorption capacity, was about 8 min: the sorption kinetics of the resin for molybdenum is very fast. In a similar way, the adsorption capacity of the cross-linked chitosan (CCTS) itself was also examined: it was found that maximum adsorption capacity for Mo (VI) was 200 mg g^{-1} . Guibal *et al* [23] reported that the adsorption capacity for Mo (VI) of chitosan (without cross linking) was about 670 mg g^{-1} . However, in our experiment,

when Mo (VI) solution was adjusted to pH 4 using 0.2 M ammonium acetate buffer, chitosan itself dissolved easily and completely. In addition, chitosan was also partially soluble in diluted hydrochloric, nitric, and sulfuric acids at pH 3 – 4. Therefore, too high adsorption capacity of Mo (VI), 670 mg g^{-1} , with raw chitosan without any cross linker is unexpected. This feature showed that the use of chitosan in raw form as solid phase extractant is restricted.

The adsorption capacity of cross-linked chitosan functionalized with leucine moiety [18] for Mo (VI) was 96 mg g^{-1} , which is much lower compared with CCTS and CCTS-NABA. This behavior can be explained as follows: the amino group ($-\text{NH}_2$) seems to play an important role for the adsorption of Mo (VI). When the cross-linker of ethyleneglycoldiglycidylether (EGDE) is chemically bonded to chitosan, the accessibility of Mo (VI) to internal site of chitosan is reduced. However, when the moiety possessed $-\text{NH}_2$ group is attached to chitosan, the adsorption capacity increases due to the extension of $-\text{NH}_2$ group, which is easily accessed by Mo (VI). This is why the adsorption capacity of CCTS-NABA is higher than CCTS. On the other hand, in case of the CCTS functionalized with leucine moiety, the extension of $-\text{NH}_2$ is not available, which results in decreasing adsorption capacity due to an increase in steric hindrance.

Adsorption behavior of metal ions on the CCTS-NABA resin

The adsorption behavior of metal ions on the CCTS-NABA resin was examined by the

column method. Figure 3 shows the results obtained for the adsorption/recovery of 10 ppb of 61 elements in the pH range from 1 to 9. The analytes adsorbed on the resin were quantitatively recovered with 10 ml of 1 M nitric acid as an eluent. The CCTS-NABA resin can adsorb various kinds of elements, such as vanadium, gallium, arsenic, selenium, silver, bismuth, thorium, tungsten, tin, tellurium, etc. However, these elements are adsorbed on the resin less than 80% at all pHs examined. Molybdenum (VI) and copper (II) could be adsorbed almost completely at appropriate pH on the resin. Copper could be adsorbed completely at neutral pH regions, whereas molybdenum could be adsorbed almost completely at acidic regions (pH 3 - 5). Accordingly, the CCTS-NABA resin is good selectivity for the adsorption of molybdenum at acidic regions and less competitive to other metal ions.

In our previous work [16], the cross-linked chitosan (CCTS) itself could adsorb copper at neutral pH region by the chelation mechanism. Similarly, in the present resin, Cu can form chelate rings with nitrogen atoms of amino groups and oxygen atoms of hydroxyl groups of the resin.

The amounts of molybdenum, which is adsorbed on the CCTS-NABA resin at acidic regions, were higher than 95%, whereas in the CCTS itself was less than 70%. As mentioned in section 3.1, the cross-linker agent (EGDE) attached to chitosan reduced the accessibility of Mo (VI) to internal site of chitosan, which can result in lower adsorption of CCTS, compared with CCTS-NABA. In addition, Mo(VI) exists as oxoanionic species in aqueous solutions,

which can be adsorbed on the resin at the acidic region by anion-exchange mechanism. By introducing NABA moiety to the CCTS, the adsorption of Mo(VI) on the CCTS-NABA resin may consist of several combination of interactions including electrostatic attraction, anion exchange and/or chelation mechanism. Compared to the CCTS itself, it can be said that CCTS-NABA resin is more effective for the adsorption/collection of molybdenum (Mo), and less competitive to other ions at acidic region. Then, pH 4 of the adsorption procedure for sample solution was selected for further experiments. Such a result resembles that coupling NABA moiety to CCTS improves selectivity toward Mo (VI).

The chemistry of molybdenum in an aqueous phase is very complex because of its multiple oxidation states and various different species [24]. Molybdenum (VI) is the most stable oxidation state in aqueous solutions. Polymeric species of molybdenum, such as $\text{H}_2\text{Mo}_7\text{O}_{24}^{4-}$, $\text{HMo}_7\text{O}_{24}^{5-}$, and $\text{Mo}_7\text{O}_{24}^{6-}$ are successively formed at pH 2.53, 3.54, and 4.40, respectively, when the concentration of Mo (VI) is greater than 96 ppm (≈ 1 mM). However, when the concentration of Mo (VI) is at several ppb levels or lower, the main species of molybdenum exist as H_2MoO_4 (pKa: 3.61), HMoO_4^- (pKa: 3.89) and MoO_4^{2-} [25]. In this work, since the collection/adsorption of molybdenum in environmental water samples was performed at pH 4, and its concentration was lower than 10 ppb, the monomeric species of MoO_4^{2-} , HMoO_4^- and H_2MoO_4 may adsorb on the CCTS-NABA resin.

Detection limit and quantification limit

The detection limit of the method (LOD) was calculated from the sum of average concentration and 3σ of blank values (LOD : blank value + 3σ , σ : standard deviation of blank value), whereas the quantification limit of the method (LOQ) was calculated from the sum of average concentration and 10σ of blank values (LOQ : blank value + 10σ). The LOD of Mo obtained by coupling the pretreatment with CCTS-NABA resin and measurement by ICP-MS was 0.01 ppb and the LOQ was 0.02 ppb when 10 ml of each sample volume and the eluent was used (without preconcentration).

The LOD of Mo using ICP-AES after pretreatment with CCTS-NABA resin was 0.19 ppb, while the LOQ was 0.40 ppb when 10-fold preconcentration of sample solution was performed.

Application of CCTS-NABA resin to the collection/concentration of molybdenum in water samples

The applicability of CCTS-NABA resin was examined for the collection/concentration of molybdenum in river water and seawater samples. The major matrices, such as Na, K, Mg and Ca can interfere with the determination of trace elements, as well as can damage the detector of the ICP-MS system. Therefore, the matrices must be removed from the samples prior to the measurement by ICP-MS. Table 1 shows the results obtained for the examination of the effect of cationic matrices, such as Na, K, Mg and Ca on the recovery of molybdenum in artificial river water and seawater samples. The results indicate that the CCTS-NABA resin is capable of adsorbing molybdenum quantitatively because almost complete recoveries (92 –

99 %) were achieved even in the presence of large amounts of matrices. The concentrations of matrices after column pretreatment were also determined by ICP-AES, and it was found that Na, K, Mg, and Ca could be removed in the range of 99 – 100%. Due to its great ability to remove matrices, the CCTS-NABA resin can be applied effectively to the collection/concentration of molybdenum in natural river water and seawater.

In order to evaluate the accuracy of the proposed method, the certified reference materials of the river water (SLRS-4), the nearshore seawater (CASS-4), and the open ocean seawater (NASS-5), issued by National Research Council Canada, were examined. The pH of the samples was adjusted to 4, followed by the procedure described in the experimental section. The results are shown in Table 2, in which there is no significant difference between the results obtained by the proposed method and the certified values. However, molybdenum in SLRS-4 could not be detected with 10-fold preconcentration by ICP-AES because its concentration is closed to the MDL of ICP-AES.

Table 3 showed the analytical results of molybdenum in river water and seawater samples obtained after the pretreatment with a mini-column packed with CCTS-NABA resin. Molybdenum in seawater can be accurately determined by ICP-AES and ICP-MS. However, 10-fold enrichment factor was required for the determination of molybdenum in river water by ICP-AES, though preconcentration procedure is unnecessary for ICP-MS.

Conclusions

The CCTS-NABA resin, synthesized by chemically bonding 3-nitro-4-amino benzoic acid (NABA) moiety to amino group of cross-linked chitosan (CCTS) through amide bond formation, provides effective separation and preconcentration of molybdenum in river water and seawater, when it is used as a packing material in the mini-column. The resin has an excellent selectivity toward molybdenum at acidic regions (pH 3-4). The molybdenum collected on the resin was easily eluted with 1M nitric acid. In addition, the CCTS-NABA resin is stable in acidic solution and can be used continuously for at least 20 cycles. Moreover, the resin offers high adsorption capacity for molybdenum (VI), 380 mg g^{-1} , which is better than other molybdenum resins reported so far [8, 18].

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Table 1 Recovery test of molybdenum in the present of matrices

Sample	Mo found / ppb		Recovery (%)	
	ICP-MS	ICP-AES	ICP-MS	ICP-AES
Artificial river water ^a	0.48 ± 0.02	0.46 ± 0.03 ^c	96	92
Artificial seawater ^b	9.88 ± 0.12	9.69 ± 0.91	99	97

All samples were adjusted to pH 4; volume of sample: 10 ml; eluent (1M HNO₃): 10 ml

^a Artificial river water contains Mo (0.50 ppb), Na (20 ppm), K (15ppm), Mg (10ppm), and Ca (50 ppm)

^b Artificial seawater contains Mo (10 ppb), Na (11500 ppm), K (1200 ppm), Mg (3900 ppm), and Ca (400 ppm).

^c Samples were concentrated by 10-fold ; volume of samples : 100 ml; eluent (1 M HNO₃) : 10 ml.

Table 2 Analytical results of molybdenum in certified reference materials

Sample	Mo found / ppb		Certified value/ ppb
	ICP-MS	ICP-AES	
SLRS-4	0.22 ± 0.00	n.d. ^a	0.21±0.02
CASS-4	8.64 ± 0.11	8.21 ± 0.44	8.78±0.86
NASS-5	9.32 ± 0.18	9.32 ± 0.28	9.6±1.0

All samples were adjusted to pH 4; volume of sample: 10 ml; eluent (1M HNO₃): 10 ml

^a Samples were concentrated by 10-fold ; volume of samples : 100 ml; eluent (1 M HNO₃) : 10 ml;

n.d. : not detected

Table 3 Analytical results of molybdenum in environmental water samples

Sample	Mo Found / ppb	
	ICP-MS	ICP-AES
River water (Asahi)	0.94 ± 0.00	0.95 ± 0.08 ^a
River water (Zasu)	0.84 ± 0.05	0.84 ± 0.01 ^a
Shibukawa seawater	9.04 ± 0.04	9.01 ± 0.20

All samples were adjusted to pH 4; volume of sample: 10 ml; eluent (1M HNO₃): 10 ml

^a Samples were concentrated by 10-fold ; volume of samples : 100 ml; eluent (1 M HNO₃) : 10 ml.

Figure

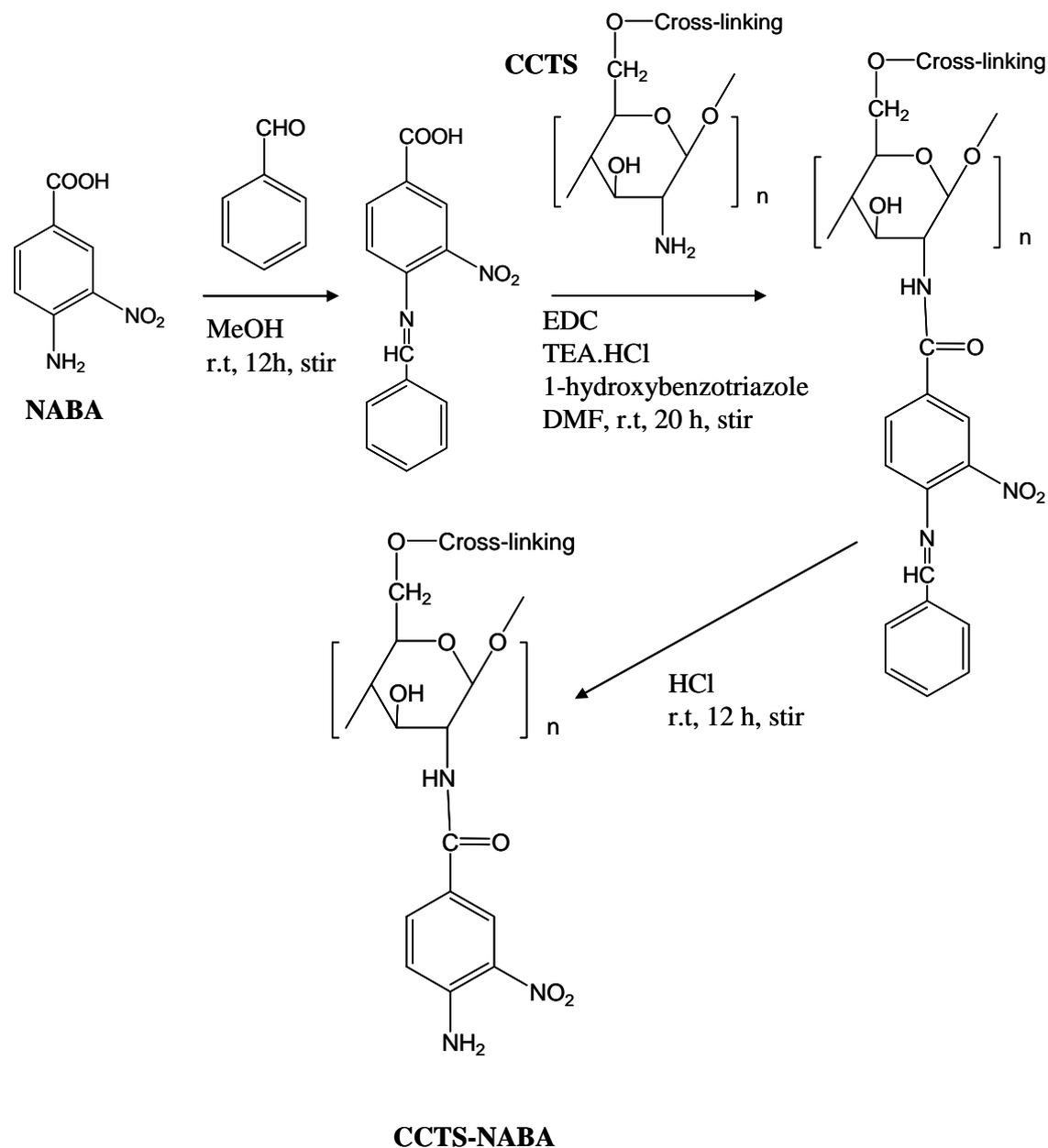
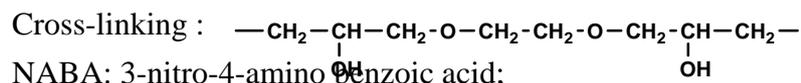


Fig. 1 Synthesis scheme of CCTS-NABA resin



NABA: 3-nitro-4-amino benzoic acid;

CCTS-NABA : cross-linked chitosan possessing 3-nitro-4-amino benzoic acid moiety;

EDC : 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide;

TEA : Triethylamine ; DMF : Dimethyl formamide.

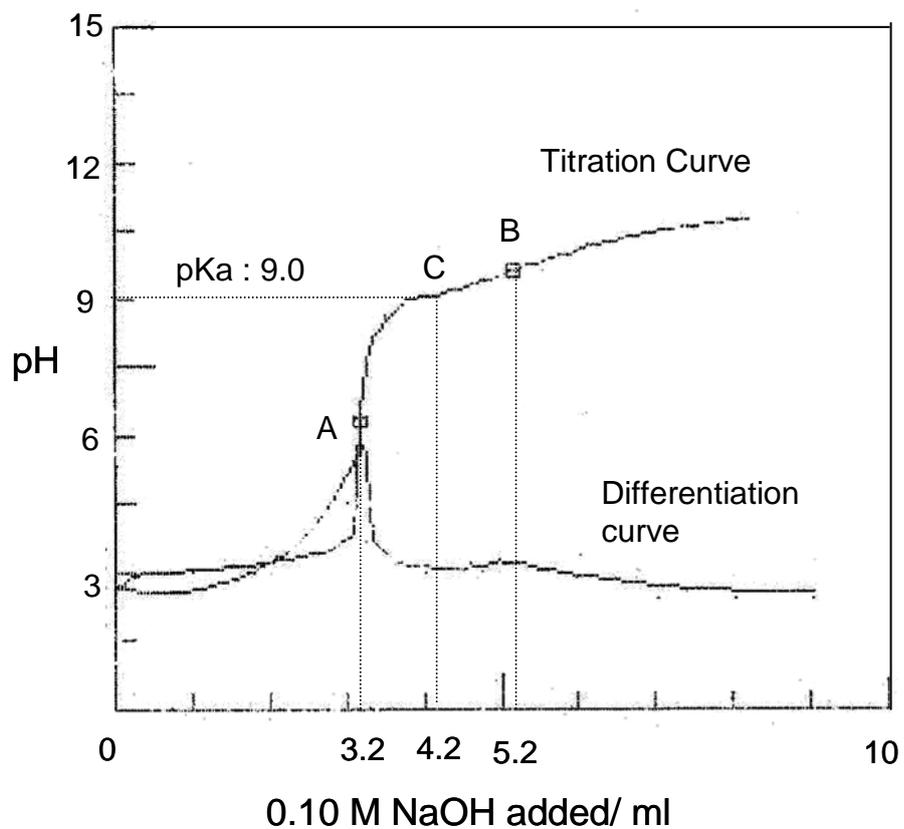


Fig. 2 Acid-base titration of the CCTS-NABA resin.

A sample for the titration consists of 0.2 g CCTS-NABA resin, 2 mL of 0.1 M HCl and 28 mL of ultrapure water;

A: the inflection point of HCl and partly protonated -NH_2 group of the resin; B: the inflection point of remaining -NH_2 in CCTS; C: the half point of the equivalent points.

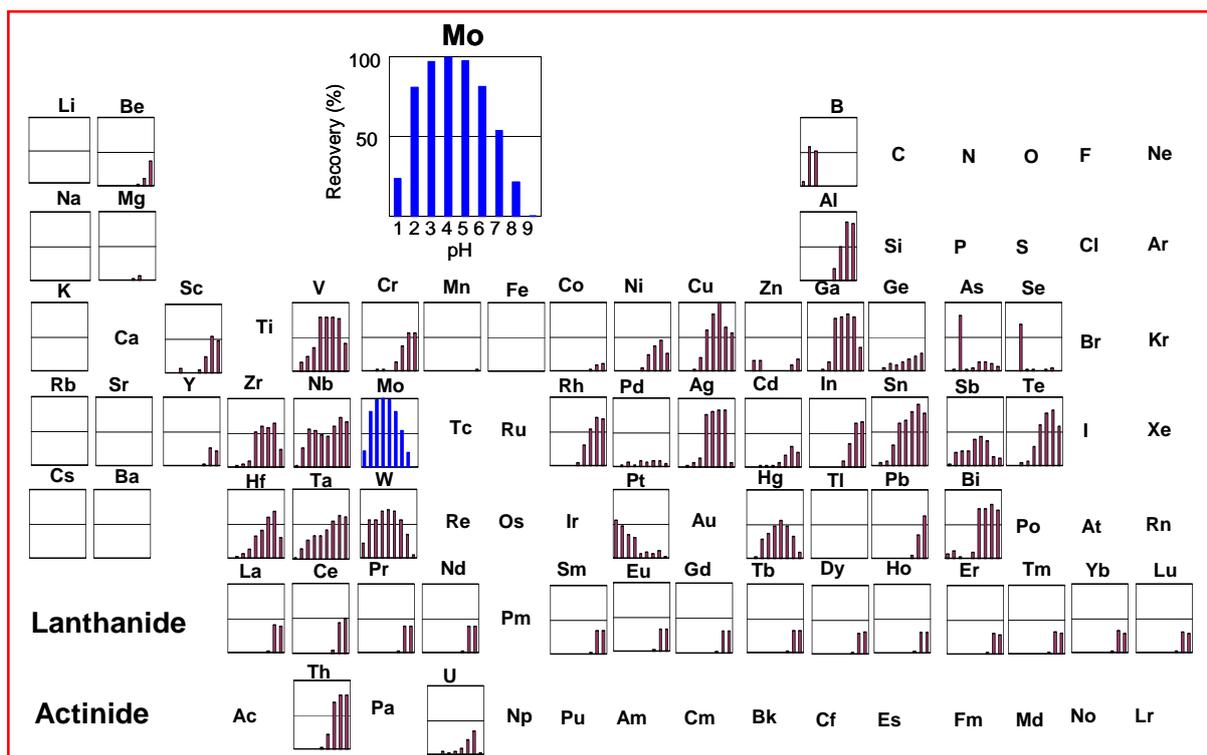


Fig. 3 Adsorption behavior of trace elements at various pHs with CCTS-NABA resin.

Sample : 10 mL; concentration of each element in the samples: 10 ppb; column: 1 mL (0.2 g dry weight) of the CCTS-NABA resin; all elements were measured by ICP-MS.