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# Synthesis of Chitosan Resin Possessing 3,4-Diamino Benzoic Acid Moiety for The Collection/Concentration of Arsenic and Selenium in Water Samples and Their Measurement by Inductively Coupled Plasma-Mass Spectrometry

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## Abstract

A chitosan resin functionalized with 3,4-diamino benzoic acid (CCTS-DBA resin) was newly synthesized by using a cross-linked chitosan (CCTS) as base material. The adsorption behavior of trace amounts of elements on the CCTS-DBA resin was examined by the pretreatment with a mini-column and measurement of the elements by inductively coupled plasma-Mass spectrometry (ICP-MS). Arsenic (V) could be retained on the CCTS-DBA resin at pH 3 as an oxoanion of $H_2AsO_4^-$. Selenium (VI) is strongly adsorbed at pH 2 and pH 3 as an oxoanion of $SeO_4^{2-}$, while selenium (IV) as $HSeO_3^-$ is adsorbed on the resin at pH 3. The sorption capacities are 82, 64, and 88 mg $g^{-1}$resin for As(V), Se(IV), and Se (VI), respectively. The effect of common anions and cations on the adsorption of As(V), Se(IV), and Se(VI) were studied; there was no interference from such anionic matrices as chloride, sulfate, phosphate, and nitrate up to 20 ppm, as well as from such artificial river water matrices as Na, K, Mg, and Ca after passing samples through the mini-column containing the resin. The CCTS-DBA resin was applied to the collection of arsenic and selenium species in bottled drinking water, tap water, and river water.

*Keywords: cross-linked chitosan, 3,4-diamino benzoic acid, arsenic, selenium, ICP-MS*

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## 1. Introduction

Arsenic and selenium are interesting elements from the viewpoint of their environmental impact. Arsenic is widespread in the environment due to its natural occurrence and former extensive use in pesticides. It is well known that inorganic arsenic species such as arsenic (III) (arsenite) and arsenic (V) (arsenate), which most commonly exist in natural water [1], are more toxic than organic arsenic compounds, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) [2-5]. World Health Organization (WHO) and United State Environmental Agency (USEPA) have set a regulation of allowable concentration for arsenic in drinking water at 10 ppb [6, 7]. Cumulative excess of arsenic in our body may cause liver, lung, kidney, bladder, or skin cancer [8]. Therefore, the trace determination of arsenic has been a growing interest because of its high toxicity in biological system, as well as environmental system. Similarly, the interest in trace determination of selenium is growing due to the dual role as an essential nutrient for humans at low concentrations and as toxic element at higher concentrations. The difference between the amounts of selenium that are beneficial and those that cause toxic effect is quite small, which means that an accurate and precise knowledge of the selenium species present in the environment is very important, and requires the monitoring of selenium level [9, 10]. In environmental samples, selenium can exist in inorganic (as elemental selenium, selenide, selenite and selenate ions) and as organic species (methylated compounds, selenoamino acids, selenoproteins and their derivative). Of these, the inorganic species, selenite and selenate, which are more toxic than organic species, are the most frequently found in natural waters and soils [11-12]. The selenium concentrations in drinking waters are regulated at less than 10 ppb in the Japan drinking water standard [13]. Recently, elemental speciation has become an important topic of research and efforts have been made to couple various separation

and preconcentration methods. It is well known that the toxicological and biological properties of most elements depend upon their chemical forms. Therefore, the knowledge on the speciation of arsenic and selenium is of particular necessity.

As far as the speciation of arsenic and selenium is concerned, an ion exchange resin and chelating resin play an important role. Basically, the ion exchange resins are used for the collection of the target species, the separation of the species, as well as the interference matrices, and the preconcentration of the species when they are present at very low levels. Commercially available resin, for example Diaion PA316, with the functional group of trymethylammonium was reported to used for the speciation of arsenic in water samples [14]. In this method, arsenic (III) is complexed with ammonium pyrrolidinedithiocarbamate (APDC) before being adsorbed on the resin, while arsenic (V) needs to be reduced to arsenic (III), and determined as total arsenic (III, V) using GF-AAS. Another resin, Muromac A-1 chelating resin with the functional groups of iminodiacetic acid, was also reported for the speciation of arsenic [15]. The Muromac A-1 chelating resin was loaded with lanthanum ion before being used as a solid-phase extraction mini-column for arsenic. Arsenic (III) and arsenic (V) were strongly adsorbed on the La-loaded chelating resin in the pH range of 4 to 9. Dambies et al. reported the adsorption of arsenic on the molybdate-impregnated chitosan beads [16] and the molybdate-coagulated chitosan beads [17]. In these methods, the arsenic sorption occurs through the complexation with molybdate ions adsorbed on the chitosan. Ion-exchange resin of Amberlite IRA-743, which has macroreticular crosslinked polystyrene matrices on which N-methyl-D-glucamine is chemically fixed, was used for the speciation of selenium by the column treatment method coupled with HPLC-ICP-MS system [18]. Anionic resin, Dowex 1X8, which has the base materials of styrene-divinylbenze functionalized with trimethyl benzyl ammonium group, was used for the speciation of selenium in plant water by a

chromatographic column method coupled with HG-AAS [19]. Other resins for the determination of selenium, such as Dionex 3589, Dowex 1-X4, Dowex 50W-X8, Dowex AG-50, Amberlite IRA-400, iron (III)-loaded Chelex 100, copper-loaded Chelex 100, etc coupled with common detection system have been reported so far [20-25]. Most of the resins for arsenic and selenium mentioned above are composed of polystyrene or poly (styrene-divinylbenzene) as base material and functional groups.

Chitosan, which possesses amino groups, is one of excellent base substances for novel resin synthesis, because it has several advantages over synthetic polymer materials like poly (styrene-divinylbenzene), polyethylene and poly urethane [26-27]. In general, the resins made of chitosan as a base materials are more hydrophilic than synthetic resins made of such base materials as polystyrene, poly (styrene-divinylbenzene), polyethylene, and polyurethane; therefore the sorption kinetics is excellent. The authors and their colleagues already reported the synthesis of a new-type cross-linked chitosan resin (EGDE cross-linked chitosan) with ethyleneglycoldiglycidylether (EGDE), which did not shrink even in a concentrated acidic solution: the adsorption behavior of metal ions on the resin were examined with a column treatment method [26, 28]. By using the EGDE cross-linked chitosan as a base material, several kinds of chelating resins possessing an iminodiacetate moiety, a serine moiety, and a leucine moiety, were synthesized and used for the collection/concentration of trace elements and their determination by ICP-MS [27, 29, 30].

In this paper, a chitosan resin derivatized with 3,4-diamino benzoic acid was newly developed by using the cross-linked chitosan as base material (CCTS-DBA resin). The adsorption behavior of trace amounts of elements on the CCTS-DBA resin was examined by the column pretreatment method. The resin could adsorb some metal cations at pH from neutral to alkaline region, and several oxoanion species, such as

arsenic (V), selenium (IV), and selenium (VI) at acidic region. Since some toxic elements adsorbed as oxo-anions on the resin, it is very interesting to examine the adsorption behavior of these elements. The CCTS-DBA resin is then applied to the collection/concentration of arsenic (V), selenium (VI), and total selenium (IV, VI) in bottled drinking water, tap water, and river water before their measurement by ICP-MS. Effects of common anionic and cationic matrices present in the samples were also examined.

## 2. Experimental

### *2.1 Reagents and materials*

Chitosan purchased from Tokyo Kasei Co. Ltd., Tokyo, Japan was a flake-type, the deacetylated degree of which was ~ 80%, and the molecular weight was ~ 1 x $10^6$. All other reagents used for the synthesis of CCTS-DBA resin were of analytical reagent grade.

The stock solution of an analytical standard for 60 elements including arsenic and selenium was prepared by diluting single element standard solutions for atomic absorption spectrometry (1000 ppm, Wako Pure Chemicals, Osaka, Japan) and a multi-element standard solution for ICP-MS (10 ppm, XSTC-13, Spex CertiPrep Inc., New Jersey, USA) with 1 M nitric acid. This stock solution was diluted with 1 M nitric acid by weight just before the column pretreatment to give a solution containing 10 ppb of each element.

To examine the speciation of arsenic and selenium, $NaAsO_2$, $Na_2HAsO_4.7H_2O$ (Wako pure chemical, Osaka, Japan), $Na_2SeO_3$, and $Na_2SeO_4$ (Sigma-Aldrich) were 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).

Ultrapure water (18.3 MΩ $cm^{-1}$ resistivity) prepared by an Elix 3/Milli-Q Element system (Nihon Millipore, Tokyo, Japan) was used throughout.

### *2.2 Instrumentations*

The ICP-MS system used for the measurement of arsenic, selenium and other elements was of Model SPQ 8000H (Seiko Instruments, Chiba, Japan). Arsenic was measured at m/z = 75, whereas selenium was measured at m/z = 78. An automatic titration system, Model GT-100 (Mitsubishi Chemical Corporation, Japan), was used for the acid-base titration of synthesized CCTS-DBA resin.

### *2.3 Procedures for the synthesis of CCTS-DBA resin*

There are two main steps in this synthesis: the first step is the synthesis of cross-linked chitosan (CCTS) with the crosslinker of ethyleneglycoldiglycidylether (EDGE), and the second step is the chemical bonding of 3,4-diamino benzoic acid (DBA) to the CCTS through the arm of chloromethyloxirane. The CCTS was synthesized in a similar manner as the previous work [28] as shown in Fig. 1 (scheme 1). The procedures are as follows: chitosan flake was ground to fine pieces and sieved to obtain chitosan particles of diameter, 100-300 μm, which were weighed (20 g) and suspended in 200 ml of ethanol. Benzaldehyde (80 g) was then added to the chitosan suspension. The mixture was stirred at room temperature for 12 h to protect amino groups of chitosan as Schiff base. After the reaction was completed, the product was filtered through glass filter and washed each 3 times with ethanol and water, respectively to remove unreacted benzaldehyde. The chitosan derivative, in

which the amino groups are protected by benzaldehyde, was refluxed with EGDE (30g) in 300 ml of dioxane and 40 ml of 1 M NaOH for 3 h. The product was filtered and washed each 3 times with ethanol and water, respectively. The Schiff base was cleaved to amino compound by twice stirring of the product in 1000 ml of 0.5 M hydrochloric acid solutions at room temperature for 12 h, followed by filtration and washing 3 times with ethanol and water, respectively.

The CCTS-DBA resin was synthesized in three steps as shown in Fig. 1 (scheme 2). In the first step, the amino groups of DBA were protected by treating 10.31 gram of DBA with 20 g of benzaldehyde in 20 ml methanol. The mixture was stirred for 20 h at room temperature. The product was filtered on the filter paper and washed with small amounts of methanol and water. The second step is the reaction of CCTS with the protected DBA, which was previously prepared. In this procedure, 16.10 g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), 11.56 g of triethylamine hydrochloride (TEA) and 1.0 g of 1-hydroxybenzotriazole were mixed with protected DBA in 40 ml dimethyl formamide (DMF). Then, 5.0 g of the CCTS was suspended in the solution; the mixture was stirred for 20 h at room temperature. The product was filtered on the glass filter and washed with methanol and water. EDC reacts first with a carboxyl group of the amino-protected DBA to form an amine-reactive intermediate. This intermediate is unstable and therefore needs 1-hydroxy benzotriazole as a stabilization reagent. Without stabilization agent, the hydrolysis of the intermediate, and the regeneration of carboxyl will occur, which results in very low yield. In the last step, the protection group (benzaldehyde), which was condensed with amino groups of DBA, 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-DBA) was filtered on the glass filter and washed with methanol and water.

### *2.4 Procedures for the column pretreatment of sample solutions*

Before being packed in the column, the CCTS-DBA resin was purified to remove residual metal impurities as follows: 5 ml of the wet resin was transferred to a 100 ml plastic beaker, containing 80 ml of 2 M nitric acid. The mixture was stirred carefully at a low speed for 6 h. The resin was then filtered on a filter paper, and rinsed with the ultrapure water. A 1 ml of the resin was then packed in polypropylene mini-columns (5 mm i.d. x 50 mm, Muromachi Chemical, Kyoto, Japan) for the examination of the collection/concentration of 60 elements.

The column pretreatment procedures are as follows: the resin, packed in the mini-columns, was washed with 10-ml aliquot of 2 M nitric acid and ultrapure water, respectively. A 5-ml aliquot of a buffer solution (pH 1-2: nitric acid; pH 3-9: 0.5 M ammonia-acetate solutions) was then passed through the column for column conditioning. A sample solution (10 ml), whose pH was adjusted with the same buffer as the one for the column conditioning, was 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 adsorbed on the resin such as alkali and alkaline earth metals. To rinse the remaining buffer components in the column, a 5-ml aliquot of the ultrapure water was passed through the column. Finally, a 10-ml of 1 mol $l^{-1}$ nitric acid was passed through the column to recover the elements adsorbed on the resin and the eluates were measured by ICP-MS.

Throughout column pretreatment, the flow rate of the sample, the rinsing solutions and the eluents, was fixed at about 1 ml $min^{-1}$. The time required for whole column pretreatment was about 1 h.

### *2.5 Procedures for the measurement of adsorption capacity*

The following procedure was used to examine the adsorption capacity of As(V),

Se(IV) and Se(VI); the sample solutions (0.1M, 100 ml) of each As(V), Se(IV) and Se(VI) were adjusted to pH 3 and placed in separate plastic beaker (100 ml).Then, 1-ml portion of the CCTS-DBA resin (0.2 g) was added to each beaker. The mixtures in the beakers were stirred, and at regular interval times, 5 ml of the solutions was pipetted and measured by ICP-MS after the appropriate dilution with 1 M nitric acid.

### *2.6 Sampling and preserving of water samples*

The bottled drinking water samples were purchased at supermarket in Okayama City. Tap water samples were collected from the faucet in the Venture Business Laboratory and faculty of science building located in Okayama University. River water samples were collected from Zasu and Asahi rivers, which flow through Okayama City. The natural pH of all samples was measured and found to be in the range of 6.8-7.0. The water samples were filtered through 0.45 μm membrane filter before introduction to the CCTS-DBA resin column and measurement by ICP-MS. Sampling and analysis were performed within the same day.

## 3. Result and discussion

### *3.1 Fundamental characteristics of CCTS-DBA resin*

Figure 2 shows the results of acid-base titration for the synthesized CCTS-DBA resin (wet volume, 1 ml; dry weight, 0.2 g) in an acidic solution (the mixture of 2 ml of 0.1 M HCl in 30 ml of ultrapure water) with a 0.1 M NaOH solution. It is considered that there are two pKa values, which can be expected from the chemical structure of CCTS-DBA resin. The pKa values come from the two amino group of DBA. For estimation of the pKa values of two amino groups of DBA, the pKa values of 1,2 diaminobenzene can be referred. The pKa values of 1,2-diaminobenzene, which are attributed to the two amino groups of that compound, are 0.80 ($pK_{a1}$) and 4.57

($pK_{a2}$)[33]. As shown in Fig.2, there are two pKa values, such as 4.88 and 8.14, were observed clearly in the titration curve. The pKa of 4.88 in CCTS-DBA resin is attributed to the amino group at *meta*-position of DBA. The pKa value of amino group at *para*-position of DBA cannot be observed at the titration curve because it is very low and may be less than 1. Probably, this group is difficult to be completely protonated by addition of strong acid: 2 ml of 0.1 M HCl was added with 28 ml of ultrapure water, and the pH was about 2.18. The pKa value of 8.14 is attributed to the amino group of CCTS remained. It means that some parts of the CCTS do not react with the DBA moiety.

As shown in titration curve (Fig.2), the first end point (A) corresponds to the neutralization of 2 ml of 0.1 M HCl and partly protonated amino group at *para*-position of DBA; that is, 2 ml of 0.1 M NaOH corresponds to 2 ml of 0.1 M HCl, and 0.595 ml of 0.1 M NaOH corresponds to about 54% of the protonated amino group at the *para*-position.

The second end point (B) corresponds to the neutralization of the protonated amino group at the *meta*-position, and 1.110 ml of 0.1 M NaOH was required. It means that 0.11 mmol of the amino group at the *meta*-position exists in 1 ml of the resin. One milliliter of the resin corresponds to 0.20 g of dry weight resin. Therefore, the amount of amino group at *meta*-position is 0.55 mmol $g^{-1}$resin. Similarly, the amino group of DBA moiety at *para*-position should be the same amount as *meta*-position, such as 0.55 mmol $g^{-1}$ resin. Then, the DBA moiety contributes amino groups content of 1.1 mmol $g^{-1}$ resin. If each DBA moiety is assumed to attach each unit of the CCTS, 2.6 mmol of amino groups may exist in 1 g of the resin. However, 1 g of the resin contains 1.1 mmol amino groups of DBA moiety. This means that the mole ratio of DBA to the unit glucamine of the CCTS is about 1:2 (= 1.1 : 2.6).

The third end point (C) corresponds to the neutralization of the protonated amino

group of unreacted CCTS. A 0.991 ml of 0.1 NaOH was required, which means that about 0.10 mmol of amino group of CCTS in 1 ml of resin (0.50 mmol $g^{-1}$resin) do not react with DBA moiety.

To obtain the adsorption capacity of CCTS-DBA, the resin (0.2 g) was equilibrated with As(V), Se(IV), and Se(VI) at pH 3 in the presence of large excess of each element. The adsorption capacities were found to be 1.1, 0.8, and 1.1 mmol $g^{-1}$resin for As(V), Se(IV), and Se(VI), respectively. Such values correspond to 82, 64, and 88 mg $g^{-1}$resin for As(V), Se(IV), and Se(VI), respectively. The adsorption capacity of CCTS itself for As(V) and Se(VI) was also examined. However, the adsorption capacity was very low and did not exceed 7 mg $g^{-1}$ resin for both species. Probably, the adsorption efficiency of As(V) and Se(VI) on the CCTS is very weak as they can easily eluted by water [31]. The adsorption time required to reach the equilibrium was within 5 min in each case. Such fast reaction rates are attributed to the hydrophilic nature of chitosan used as base material.

As mentioned above that the amino groups of DBA moiety attached to CCTS was 1.1 mmol $g^{-1}$ resin, whereas the adsorption capacities of CCTS-DBA resin were 1.1, 0.8, and 1.1 mmol $g^{-1}$resin for As(V), Se(IV), and Se(VI), respectively. From these results, apparently, the stoichiometric ratio between amino groups of DBA moiety and arsenic or selenium species is about 1 : 1.

### *3.2 Adsorption behavior of 60 elements on the CCTS-DBA resin*

The adsorption behavior of metal and non-metal ions was examined by the column method as shown in the procedure 2.4. Figure 3 shows the results obtained for the adsorption/recovery of 10 ppb for the 60 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-DBA resin can adsorb various kinds of elements, such

as vanadium, molybdenum, copper, gallium, arsenic, selenium, silver, indium, bismuth, and thorium. Of these, gallium could be adsorbed at neutral to alkaline pH region, whereas bismuth, indium and thorium could be adsorbed only at alkaline pH region. Silver, copper, and vanadium could be adsorbed from acidic to alkaline pH regions, whereas arsenic and selenium could be adsorbed only at acidic pH region. Molybdenum could be adsorbed from acidic to neutral regions. Such analytes could be adsorbed almost completely at appropriate pH on the resin and could be easily recovered with diluted nitric acid.

In the previous work reported by Motomizu *et.al*, the cross-linked chitosan (CCTS) itself could adsorb copper at neutral pH region by the chelation mechanism [28]. Similarly, in the present resin, Cu can form five-membered chelate rings with nitrogen atoms of amino groups and oxygen atoms of hydroxyl groups of chitosan. In the CCTS-DBA resin, oxo-anions, such as vanadium, molybdenum, gallium, and bismuth, can adsorb on the resin by an anion exchange mechanism, as they can adsorb on the CCTS itself [16, 27, 28, 30, 31]. Such adsorption behavior suggests that the DBA moiety attached to the CCTS is very useful for the adsorption of silver and other oxo-anions, such as arsenic, and selenium. Compared to the CCTS itself, it can be said that functional groups of DBA in the CCTS-DBA resin is effective for the adsorption/collection of silver, arsenic, and selenium and less competitive with other ions at acidic region. It is very interesting to investigate the adsorption behavior of arsenic and selenium on the CCTS-DBA resin with respect to the speciation of arsenic and selenium because of their toxicological effect in the environment. In addition, the speciation is important for determining the sorption selectivity for metal recovery and other analytical purposes.

### *3.3 Speciation of arsenic and selenium*

As mentioned above, the CCTS-DBA resin is selective for the adsorption of arsenic and selenium. To investigate the adsorption behavior in more detail, the speciation of As(III) and As(V), and Se(IV) and Se(VI) was performed by using the mini-column containing 1 ml of wet resin.

The speciation of As(III) and As(V) can be conducted at pH 3. As shown in Fig. 4(a), As (V) could be adsorbed at around pH 3 on the resin and easily eluted with 1 M nitric acid and the recovery of 99.7% was obtained, whereas at pH 3, As(III) could not be retained on the resin. At around pH 3, As (V) exists in the form of a deprotonated oxoanion ($H_2AsO_4^-$ ; $pK_{a1}$ : 2.2), while As(III) exist as a protonated form ($H_3AsO_3$, $pK_{a1}$ : 9.2). Also, a mixed sample containing 10 ppb of each As(III) and As (V) was treated with CCTS-DBA column after adjusting the pH to 3. The result showed that the recovered amount of arsenic was 9.96±0.03 ppb, which implies that one arsenic species, As (III), was not retained on the resin. The adsorption mechanism of As(V) on the CCTS-DBA resin may consist of one or combination of several reactions including electrostatic attraction, ion exchange and/or chelation. At present, however, it is not clear, which mechanism operates in the CCTS-DBA resin.

The adsorption of Se(IV) and Se(VI) on the resin was also examined by using a single solution of each species. As shown in Fig 4(b), the adsorption of Se(IV) and Se(VI) can be performed at pH 2 and pH 3. The species of Se(VI), which has $pK_{a2}$ of 1.9, could be adsorbed on the resin at pH 2, whereas Se(IV), which has $pK_{a1}$ of 2.6, was not retained on the resin. The recovery of Se(VI) at pH 2 was more than 99%. However, at pH 3, both species of selenium could be adsorbed on the resin; the recovery of both species were 101.3% and 101.2% for Se(IV) and Se(VI), respectively. The mixed samples containing 10 ppb of Se(IV) and Se(VI) species were also treated with the CCTS-DBA column. The samples were adjusted to pH 2 and pH 3. The

concentration of selenium found at pH 2 was 9.92±0.03 ppb, which corresponds to Se(VI) and that found at pH 3 was 20.14±0.02 ppb, which corresponds to the total amount of Se(IV) and Se (VI). From these results, the CCTS-DBA resin can be used effectively for the speciation of selenium using appropriate pH conditions; that is pH 2 for Se(VI) and pH 3 for the total of selenium (IV) and (VI). At pH 3, the two species exist in the form of $HSeO_3^-$ and $SeO_4^{2-}$ for Se(IV) and Se(VI), respectively. Selenium is well adsorbed on the DBA moiety of the resin. This is probably due to the adsorption by the complexation mechanism. The adsorption mechanism of selenium on the resin was also assumed in the previous work [32], which reported that selenium could form benzoselenadiazole complex using two amino groups of 1,2-diaminobenzene.

### *3.4 Effect of cation and anion matrices*

The matrices, such as Na, K, Mg and Ca, in artificial river water samples can interfere with the determination of trace elements. The alkali and alkaline earth metal matrices can easily release electron in the plasma. When the sample containing elements of interest is introduced to the plasma, it can capture electron released by the matrices, which results in higher atomic state and lower ionic state of the target elements. Since the target elements are measured as ionic state, their concentration will be found in lower amounts than their actual concentration. 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 cation matrices, such as Na, K, Mg and Ca, on the recovery arsenic and selenium species. The cation matrices were prepared from nitrate salt of each element. The results indicated that the CCTS-DBA resin is capable of adsorbing arsenic and selenium species quantitatively even in the presence of high cation matrices, whereas almost all amounts of the alkali and alkaline earth metal ions could be removed after the column

pretreatment. These results indicated that the CCTS-DBA resin has potential to be applied as separation column to separate As (V), Se(IV), and Se(VI) from the cation matrices in sample solution.

Table 1 also shows the results obtained from the examination of the effect of arsenic and selenium species present at various concentrations in the sample solutions on their recoveries with 10 ml of 1 M nitric acid as an eluent. In the wide concentration range of each species, the CCTS-DBA resin could adsorb As (V), Se(IV), and Se(VI) quantitatively with excellent recoveries.

Inductively coupled plasma-mass spectrometry (ICP-MS) is widely used for the determination of arsenic and selenium because the detection limits of ICP-MS are sufficient for the accurate determination of low level of these elements present in environmental samples. However, the determination of arsenic and selenium by quadrupole-based ICP-MS is problematic for many samples as the isotopes of both elements typically suffer from severe spectral interferences. For example, in the sample with high chloride concentration, polyatomic ion of $^{40}Ar^{35}Cl$ interferes with the determination of $^{75}As$. Therefore, effect of chloride as well as common anions, viz., phosphate, sulfate, and nitrate were examined in the range 0 to 20 ppm. No interference was found in the range concentration examined as shown in Table 2.

### 3.5 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was calculated from the sum the concentration of reagent blank and 3σ of reagent blank (LOD : blank + 3σ , σ : standard deviation of reagent blank), while LOQ was calculated from the sum of the concentration of reagent blank and 10σ (LOQ : blank + 10σ). The LOD of arsenic was 0.04 ppb, while the LOQ was 0.09 ppb when 10 ml of each sample solution and eluent was used. The LOD of selenium found was 0.20 ppb, while the LOQ found was 0.39 ppb when 10 ml of each sample solution

and eluent was used.

### 3.6 Accuracy of the method

In order to evaluate the accuracy of the developed procedure, certified reference material for river water such as JAC 0031 and JAC 0032 (Japan Society for Analytical Chemistry), were analyzed. The results are given in Table 3. It was found that there is no significant difference between attained result by the proposed method and the certified value.

### *3.7* Application of CCTS-DBA resin to water samples

The CCTS-DBA resin was applied as column pretreatment for the collection/ concentration of As (V), Se(VI) and total Se (IV, VI) from bottled drinking water, tap water, and river water. As (V) and total Se (IV, VI) could be collected at pH 3, while Se(VI) could be adsorbed at pH 2. Arsenic in bottled drinking water, tap water, and river water were present above the LOD and LOQ of ICP-MS, so that the preconcentration procedure was not required, and arsenic could be determined accurately with this instrument. In the case of selenium, bottled drinking water, tap water river water samples contained selenium below the LOD and LOQ of ICP-MS. Therefore, the samples were concentrated by 10-fold. The analytical results for the determination of As(V), Se(VI) and total Se (IV, VI) in water samples after pretreatment with CCTS-DBA resin are summarized in Table 4. The results indicate that Se (VI) seem to be predominant in all water samples.

## 4. Conclusions

The 3,4-diamino benzoic acid (DBA) moiety bound to cross-linked chitosan (CCTS), gave a good adsorption for As(V) at pH 3, Se (VI) at pH 2, total Se (IV, VI) at pH 3

accompanied by excellent recoveries using 1 M nitric acid. The sorption capacities are 0.22 mmol $ml^{-1}$ for each As(V) and Se(VI), while 0.17 mmol $ml^{-1}$ for Se (IV). The other advantages of this resin include; (1) quantitative collection/adsorption of arsenic and selenium, (2) no interference from anionic matrices viz., chloride, sulfate, phosphate, and nitrate up to 20 ppm, as well as from artificial river water matrices containing Na, K, Mg, Ca after passing sample through small resin column, (3) no loss activity for long term uses (at least 20 times), (4) effective collection, with a mini column (1 ml resin) and subsequent easy elution with 1 M nitric acid, of arsenic and selenium contained in bottled drinking water, tap water, river water.

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Table 1 Recovery of As (V), Se(IV) and Se(VI) adsorbed on the CCTS-DBA resin

| Substance | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0.5 ppb | | 1 ppb | | 10 ppb | |
| | Standard [b] | Artificial river water [c] | Standard [b] | Artificial river water [c] | Standard [b] | Artificial river water [c] |
| As (V) pH 3 | 99.8±0.6 | 99.8±1.3 | 99.9±0.6 | 100.0±1.4 | 99.7±0.4 | 99.4±0.6 |
| Se (IV) pH 3 | 100.8±0.7 | 100.5±3.4 | 100.3±0.5 | 100.4±1.5 | 101.2±0.6 | 100.6±1.0 |
| Se (VI) pH 3 | 101.0±1.0 | 100.9±1.4 | 100.9±0.2 | 100.9±1.4 | 101.3±0.4 | 101.8±2.3 |
| Se (VI) pH 2 | 100.0±0.1 | 100.1±1.8 | 99.6±0.8 | 101.1±1.6 | 99.2±0.7 | 99.2±1.8 |

[a] Samples, 10 ml, were treated with the column (CCTS-DBA resin: 1 ml). Eluents, (1 M $HNO_3$): 10 ml. The eluents were measured by ICP-MS.

[b] without addition of Na, K, Mg, and Ca matrices to the samples

[c] The cation matrices of Na, 20 ppm; K, 10 ppm; Mg, 15 ppm; Ca, 50 ppm, were added to the sample

All cations were prepared from nitrate salts.

Samples were measured from average of three measurements

Samples were prepared from single standard of each element.

Table 2 Recovery of As(V), Se(IV), and Se (VI) at pH 3 adsorbed on the CCTS-DBA in the presence of anion matrices

| Mixed anion added [a] / ppm | Recovery (%) [b] | | |
|---|---|---|---|
| | As (V) [c] | Se(IV) [c] | Se(VI) [c] |
| 0 | 99.7±0.4 | 101.2±0.6 | 101.3±0.4 |
| 1 | 97.2±1.3 | 101.2±4.4 | 102.6±2.1 |
| 5 | 99.6±3.7 | 101.3±6.4 | 101.5±1.1 |
| 10 | 99.8±5.0 | 101.6±1.0 | 100.5±2.1 |
| 20 | 100.0±0.7 | 100.8±6.1 | 102.0±2.7 |

[a] Mixed-solution containing anions of $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$, $NO_3^{2-}$ were added to the samples; all anions were prepared from sodium salts.

[b] Samples, 10 ml, were treated with the column (CCTS-DBA resin: 1 ml). Eluents, (1 M $HNO_3$): 10 ml. The eluents were measured by ICP-MS.

[c] Samples were prepared from single standard of each element with the concentration of 10 ppb

Table 3 Analysis of river water standard reference materials

| Sample | As / ppb [a] | | Se total / ppb [a] | | Se (VI) / ppb [b] | |
|---|---|---|---|---|---|---|
| | Found [c] | Certified | Found [d] | Certified | Found | Certified |
| JAC 0031 | 0.23±0.01 | 0.28±0.04 | n.m | (0.1) [e] | n.m | - |
| JAC 0032 | 5.06±0.02 | 5.5±0.3 | 5.06±0.01 | 5.2±0.03 | 4.41±0.05 | - |

[a] Samples were adjusted to pH 3

[b] Samples were adjusted to pH 2

[c] Concentration of As (V)

[d] Concentration of Se (IV, VI)

[e] Information only

n.m : not measured

Table 4 Analytical results for the determination of As(V), Se(IV, VI), and Se (VI) in water samples

| Sample | As (V) / ppb [a] | | | Se (IV, VI) / ppb [a,c] | | | Se (VI) / ppb [b,c] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Added [d] | Found | Recovery (%) | Added [e] | Found | Recovery (%) | Added [e] | Found | Recovery (%) |
| Drinking water A (Volvic) | 0 | 4.051±0.068 | | 0 | 0.080±0.003 | | 0 | 0.080±0.003 | |
| | 4.998 | 8.965±0.024 | 98.3 | 0.052 | 0.133±0.010 | 101.9 | 0.051 | 0.130±0.004 | 98.0 |
| Drinking water B (Vitel) | 0 | 5.489±0.113 | | 0 | 0.058±0.001 | | 0 | 0.059±0.002 | |
| | 5.022 | 10.283±0.453 | 95.5 | 0.053 | 0.102±0.009 | 83.0 | 0.052 | 0.109±0.006 | 96.1 |
| Tap water A (VBL) | 0 | 1.005±0.034 | | 0 | 0.068±0.002 | | 0 | 0.067±0.006 | |
| | 0.506 | 1.460±0.006 | 89.9 | 0.053 | 0.119±0.016 | 96.2 | 0.052 | 0.118±0.004 | 98.1 |
| Tap water B (Fac. of Science) | 0 | 0.195±0.005 | | 0 | 0.041±0.001 | | 0 | 0.044±0.005 | |
| | 0.512 | 0.697±0.009 | 98.0 | 0.053 | 0.091±0.003 | 94.3 | 0.052 | 0.095±0.012 | 98.1 |
| River water A (Zasu) | 0 | 0.356±0.035 | | 0 | 0.238±0.028 | | 0 | 0.215±0.006 | |
| | 0.509 | 0.854±0.007 | 97.8 | 0.220 | 0.449±0.022 | 95.9 | 0.216 | 0.426±0.010 | 97.7 |
| River water B (Asahi) | 0 | 0.226±0.005 | | 0 | 0.150±0.010 | | 0 | 0.123±0.012 | |
| | 0.514 | 0.731±0.011 | 98.2 | 0.225 | 0.366±0.010 | 96.0 | 0.220 | 0.348±0.013 | 102.3 |

[a] Samples were adjusted to pH 3; [b] samples were adjusted to pH 2; [c] samples were concentrated by 10-fold ; volume : 100 ml; [d] spiked with As (V); [e] spiked with Se (VI); all samples were measured by ICP-MS

## Figures

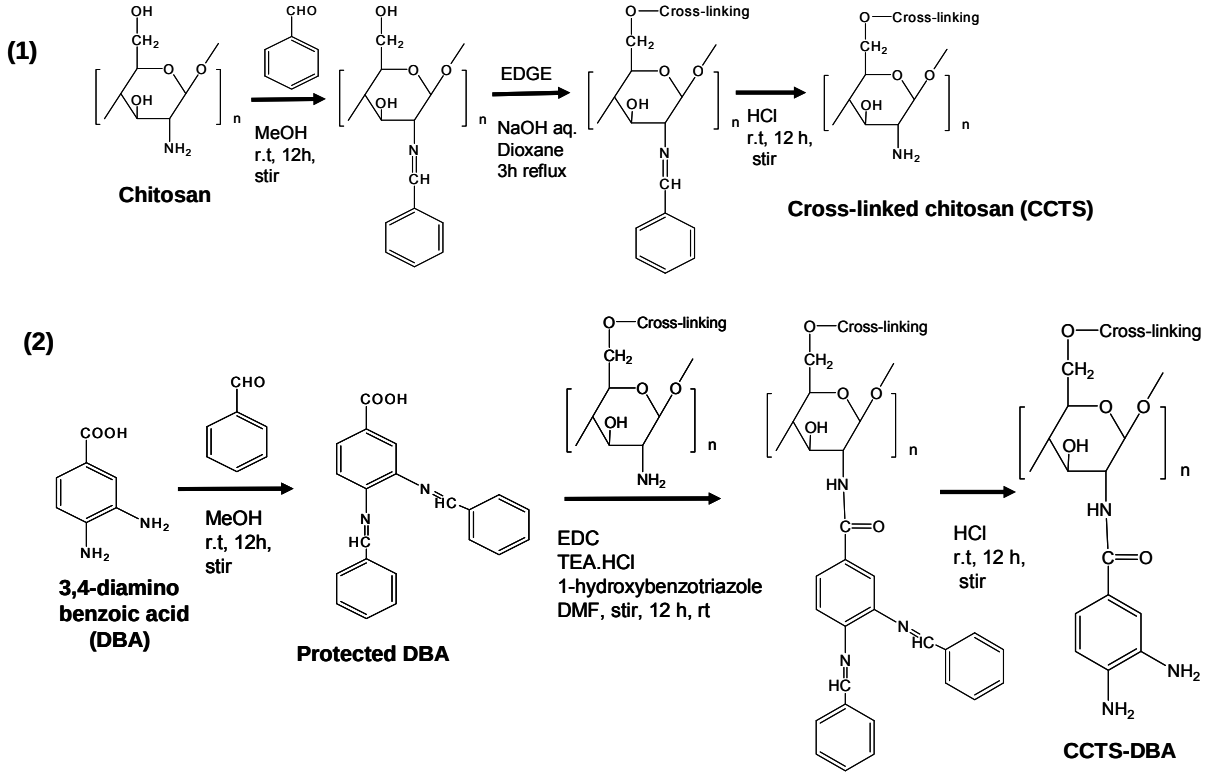
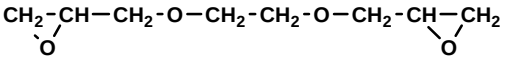
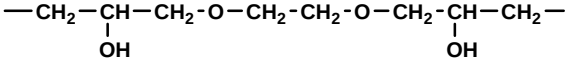



Fig. 1 Scheme for the synthesis of CCTS-DBA

EDGE :



Cross-linking :



CCTS-DBA : cross-linked chitosan possessing 3,4-diamino benzoic acid moiety;
EDC : 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide;
TEA.HCl : Triethylamine hydrochloride ; DMF : Dimethyl formamide.

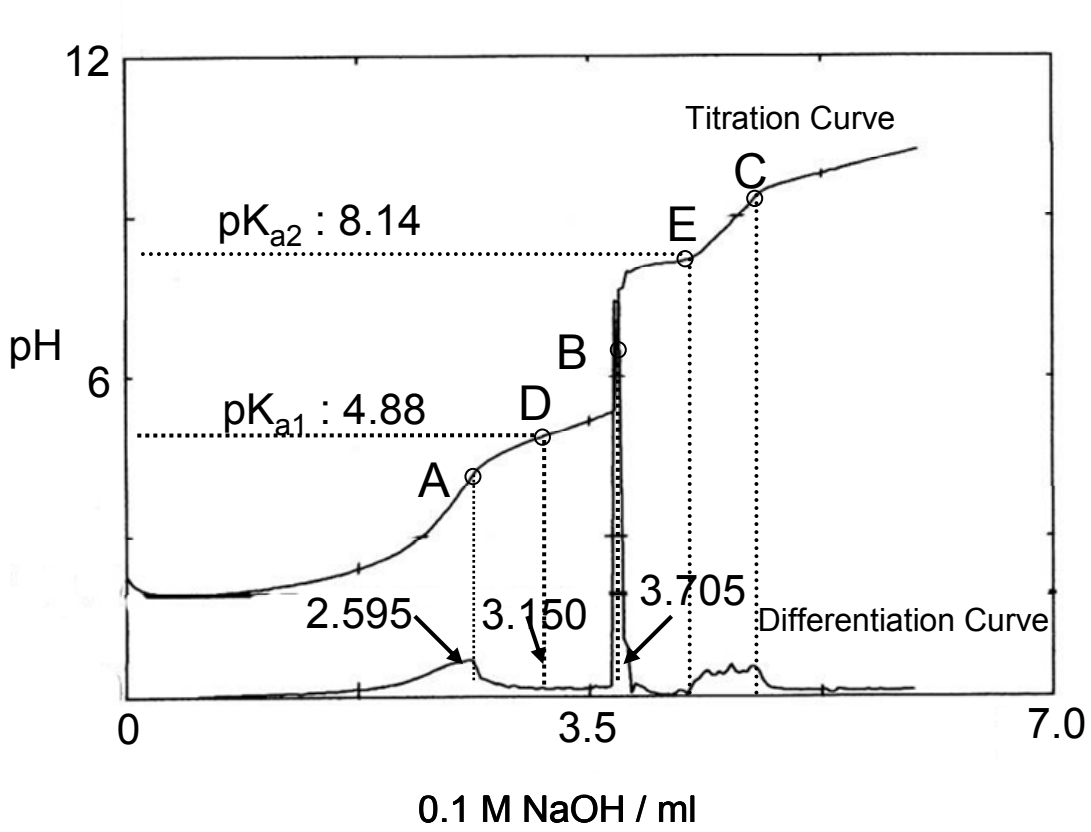



Fig.2 Acid-base titration curve of the CCTS-DBA resin.
A sample for the titration consists of 1 ml of the CCTS-DBA resin (wet volume, 0.2 dry weight), 2 ml of 0.1 M hydrochloric acid and 28 ml of the ultrapure water;
A: the inflection point of hydrochloric acid; B, C: the inflection point of CCTS-DBA resin; D, E: the half point of the equivalent points.

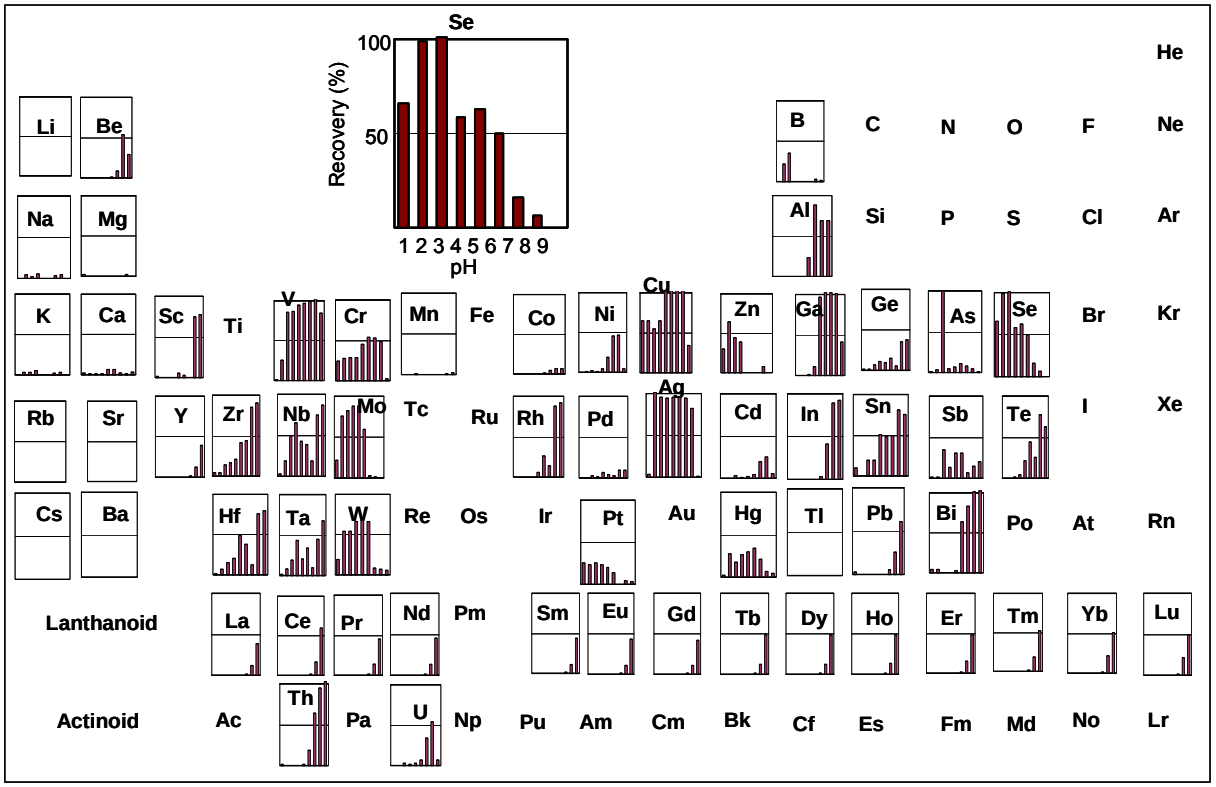



Fig. 3 Adsorption behavior of trace elements at various pHs with CCTS-DBA resin. Sample : 10 ml; concentration of each element in the samples: 10 ppb; column: 1 ml of the CCTS-DBA resin (0.2 dry weight).

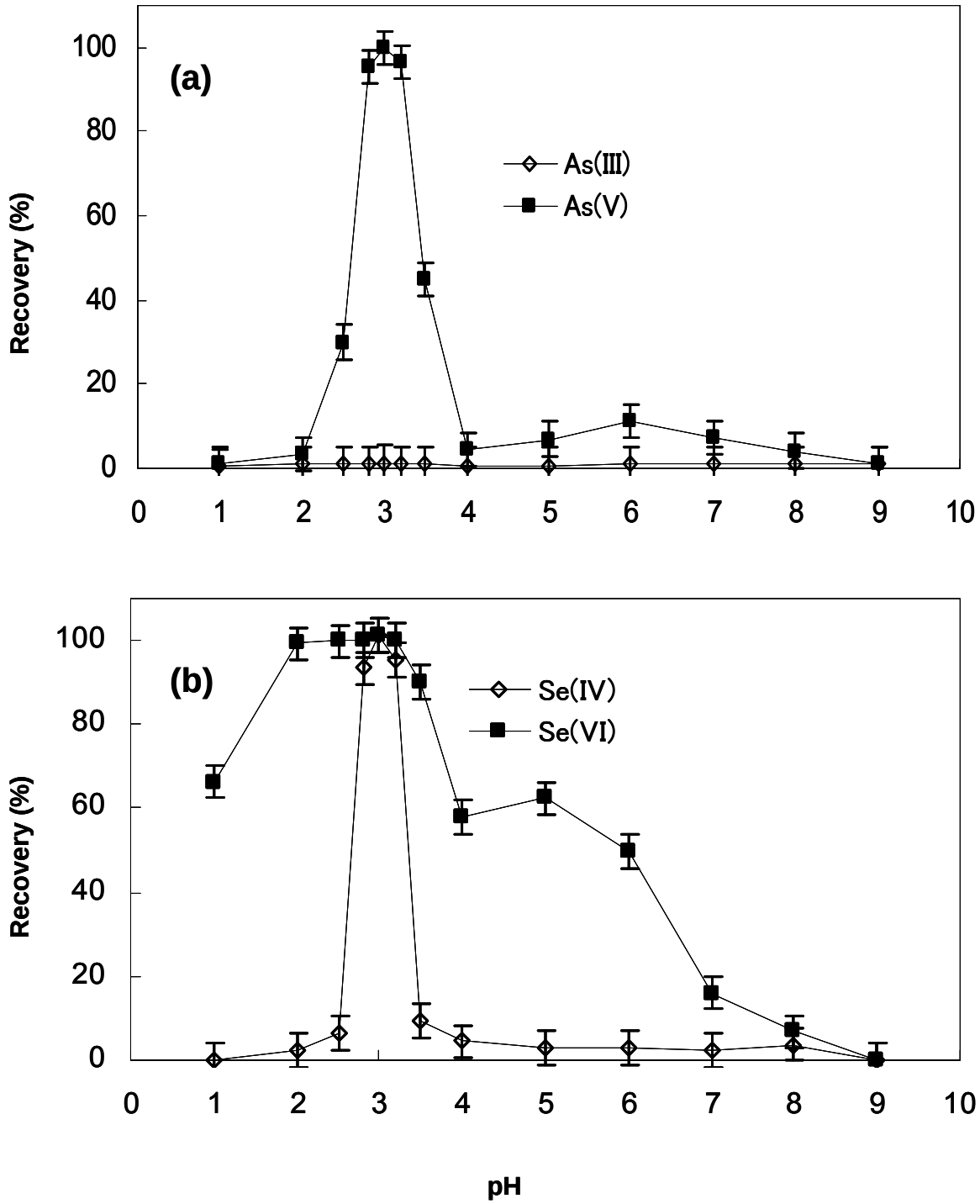



Fig 4 Speciation of arsenic and selenium
Column : 1ml of CCTS-DBA resin ( wet volume, 0.2 g dry weight);
Sample : single element of As (III), As (V), Se(IV), and Se(VI) with each concentration of 10 ppb. Error bars : ±95% confidence interval