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# Functionalization of Chitosan with 3,4-Dihydroxybenzoic Acid for The Adsorption/Collection of Uranium in Water Samples and Its Determination by Inductively Coupled Plasma-Mass Spectrometry

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## Abstract

A chitosan resin derivatized with 3,4-dihydroxybenzoic acid moiety (CCTS-DHBA resin) was newly synthesized for the collection/concentration of trace uranium by using cross-linked chitosan (CCTS) as base material, and the adsorption behavior of uranium as well as 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 collected elements on the resin with 1 M HNO<sub>3</sub>, the eluates were measured by inductively coupled plasma-mass spectrometry (ICP-MS).

The CCTS-DHBA resin can adsorb several metal cations and several oxoanionic elements at appropriate pH. Among these metal ions, uranium shows an excellent adsorption behavior on this resin. Uranium as UO<sub>2</sub><sup>2+</sup> species can be adsorbed on the resin by chelating mechanism with adsorption capacity of 330 mg g<sup>-1</sup> resin. Through the column treatment, the complete removal of large amounts of alkali and alkaline earth matrices without any loss of adsorption efficiency over prolonged usage were achieved with this resin.

The CCTS-DHBA resin was applied to the adsorption/collection of uranium in tap water, river water and seawater samples with satisfactory results. The validation of the proposed method was carried out by analyzing uranium in the standard reference materials of SLRS-4, CASS-4, and NASS-5 after passing through the CCTS-DHBA resin, and the results showed good agreement with the certified values.

*Keywords: chitosan resin, 3,4-dihydroxybenzoic acid, uranium, adsorption, water, inductively coupled plasma-mass spectrometry*

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## 1. Introduction

Currently, the intense interest in uranium arises from its known toxicity and the possibility of human exposure to it. Such exposure can cause acute renal failure resulting in a fatality or minor damage to the liver. Hence, World Health Organization (WHO) [1], Health Canada [2], National Health and Medical Research Council (NHMRC) of Australia [3], and United State Environmental Protection Agency (USEPA) [4] have regulated maximum concentration level of uranium in drinking water to be 15, 20, 20, and 30 ng mL<sup>-1</sup>, respectively. In order to investigate the behavior of uranium in aquatic environment, collection and concentration methods are prerequisite for the determination of uranium at low concentration level in natural aquatic environment. The concentration of uranium in sea water is about 3 ng mL<sup>-1</sup>, which can be easily detected by inductively coupled plasma-mass spectrometry (ICP-MS), since the limit of detection (LOD) of this instrument is about 0.001 ng mL<sup>-1</sup>. However, one of the main limitations of this technique is the need for sample preparation prior to analysis, as high levels of matrix components, such as Na, K, Ca, Mg, can give serious damage to mass spectrometer and the deposition of matrix constituents on the sampler and skimmer cones of the spectrometer, which can seriously affect the accuracy of analytical results. Therefore, the matrices must be removed from the sample solutions before measurement by ICP-MS. In addition, the preconcentration procedure is necessary when uranium exist at extremely low level to achieve more accurate measurement. In order to overcome these problems, ion exchange resins, as well as chelating resins, have been used so far.

Rao et al. [5] reported Merrifield chloromethylated resin anchored with quinoline-8-ol, which

has high adsorption capacity of uranium about  $120 \text{ mg g}^{-1}$  resin. It also provides fast kinetics rate since 90% of  $1 \text{ } \mu\text{g mL}^{-1}$  uranium can be adsorbed within 2 min. However, the resin only offers adsorption of uranium at critical pH regions of 5.0-6.0. Another Merrifield chloromethylated resin anchored with di-bis(2-ethylhexyl)malonamide [6] resulted in notable features for uranyl ion-selectivity, and rapid attainment of phase equilibration, in which more than 99.5% of  $10 \text{ } \mu\text{g mL}^{-1}$  uranium was adsorbed within less than 15 min. The sorption capacity for uranium was found to be  $62.5 \text{ mg g}^{-1}$  resin. The modified Amberlite XAD-16 (a styrene-divinyl benzene copolymer) incorporated with *N,N*-dibutyl-*N'*-benzoylthiourea [7] showed a good ability for the adsorption of uranium. This resin provides an excellent uranium retention-sorption capacity of  $214.20 \text{ mg g}^{-1}$  resin. However, the moiety is physically loaded on the polymeric matrix, and the lower stability of the resin may offer non-quantitative recoveries. The commercially available chelating resins, UTEVA resin possessing dipentylpentylphosphonate (DPPP) [8-10], and anion-exchange resin, such as Dowex 1-X8 [11], can adsorb uranium only in concentrated acidic solutions. Such resin produces a large amount of acidic wastes, which is one of the serious problems from environmental point of view.

Chitosan, which possesses amino groups, is an excellent base material for novel resin synthesis. The use of chitosan as solid support for ion exchange and chelating resins is of great interest due to its advantages, such as easy derivatization of its amino group and more hydrophilic than synthetic base materials like polystyrene-divinylbenzene, polyethylene and polyurethane, which provides fast sorption kinetic of species. Atia [12] reported functionalized

cross-linked chitosan with ethylenediamine and 3-amino-1,2,4-triazole-5-thiol for the adsorption of uranium and mercury. Although the adsorption capacity of uranium is very high ( $404 \text{ mg g}^{-1}$ ), however, it should be performed at  $30^\circ\text{C}$  which is not convenient from practical view point. The authors have successfully synthesized two chitosan resins possessing serine and imino di(methyl phosphonic acid) (IDP) moieties for the adsorption/collection of uranium in aqueous samples [13-14]. Uranium collected on the IDP-type chitosan resin is very difficult to be eluted even with high concentration of acid, such as  $6 \text{ M HNO}_3$  or  $6 \text{ M HCl}$ . On the other hand, uranium collected on the serine-type chitosan resin is easily eluted with  $1 \text{ M HNO}_3$ .

In this work, new chitosan resin for adsorption/collection of uranium was developed. The moiety of 3,4-dihydroxy benzoic acid was chemically bonded to the amino group of cross-linked chitosan (CCTS-DHBA resin) through the amide bond formation. The newly developed resin is superior in term of sorption capacity to the serine-type chitosan resin, as well as to other uranium resins reported so far. Moreover, the synthesis procedure is much simpler than other functionalized resins. The collected uranium on the CCTS-DHBA resin is easily eluted with  $1 \text{ M}$  nitric acid. The resin can be applied to the adsorption/collection of uranium in tap water, river water, and seawater samples before its measurement by ICP-MS. The matrices commonly existing in river and seawater samples were successfully removed by using CCTS-DHBA resin.

## **2. Experimental**

### ***2.1 Reagents and Materials***

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

Working solution of multielement standard solutions were prepared by diluting several kinds of a single element standard solution for atomic absorption spectrometry ( $1000 \mu\text{g mL}^{-1}$ ) purchased from Wako Pure Chemicals (Osaka, Japan) and a multi-element standard solution for ICP-MS; XSTC-13 and XSTC-1 ( $10 \mu\text{g mL}^{-1}$ ) provided by Spex CertiPrep Inc. (Metuchen, New Jersey, USA). The XSTC-13 containing 31 elements of 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, whereas XSTC-1 consist of 16 elements, namely Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Tm, Yb, and Y. These solutions were mixed and diluted with 1 M nitric acid by weight just before the column pretreatment to give a solution containing  $10 \text{ ng mL}^{-1}$  of each element.

Ultrapure grade nitric acid (60 %, density  $1.38 \text{ g mL}^{-1}$ ) from 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 purchased from Kanto Chemicals (Tokyo, Japan).

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

## ***2.2 Instrumentations***

The ICP-MS used was a SPQ 8000H, Seiko Instruments (Chiba, Japan), equipped with a glass concentric nebulizer model TR-30-C2, J.E. Meinhard Associates, Inc. (Santa Ana, California, USA). Uranium was measured at  $m/z = 238$ . The operating conditions of ICP-MS were similar to those reported in our previous work [22]. Infrared spectra ( $4000\text{-}400\text{ cm}^{-1}$ ) were taken by 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-DHBA resin.

## ***2.3 Synthesis of CCTS-DHBA resin***

The cross-linked chitosan (CCTS) with the cross-linker of ethyleneglycoldiglycidylether (EGDE) was synthesized in similar manner to the previous work [13-18]. Then, the CCTS-DHBA resin was synthesized by reacting CCTS with 3,4-dihydroxy benzoic acid (DHBA) through the amide bond formation as shown in Fig. 1. In this procedure, 10 g of DHBA, 16 g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), 12 g of triethylamine (TEA) and 1 g of 1-hydroxybenzotriazole were mixed with DHBA in 60 mL of dimethyl formamide (DMF). Then, 5 g of the CCTS was suspended in 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. EDC reacts first with carboxyl group of the DHBA moiety to form an amine-reactive intermediate. This intermediate is unstable and therefore, 1-hydroxy

benzotriazole is required as a stabilizing reagent. Without stabilizing agent, the hydrolysis of the intermediate and the regeneration of carboxyl can occur, which results in very low yield.

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

Before being packed in the column, the CCTS-DHBA resin was washed to remove residual metal impurities during synthesis 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 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 collection/concentration of trace elements.

The column pretreatment procedures are similar to our previous work [13-18, 21-22]. 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 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 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-MS.

Throughout the column pretreatment, the flow rate of the sample, other solutions including the eluents, was fixed at about  $1 \text{ mL min}^{-1}$ . The time required for whole column pretreatment was about 1 h.

## ***2.5 Samples***

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

Tap water sample was collected from the faucet in the Venture Business Laboratory Building located in Okayama University. River water samples were collected from Zasu and Asahi River, which flows through Okayama City. Seawater samples were sampled at Shin Okayama Port and Shibukawa located in Okayama Prefecture. The pH of all samples was found to be in the range of 6.5-7.3. These samples were acidified to about pH 1 by adding concentrated nitric acid before filtration with  $0.45 \mu\text{m}$  membrane filter. Sampling and analysis were performed within the same day.

## **3. Results and discussion**

### ***3.1 Characteristics of CCTS-DHBA resin***

The IR absorption frequency assignment to the CCTS-DHBA resin was  $1708.64 \text{ cm}^{-1}$ . This

frequency depicted the additional bands compared to CCTS or chitosan itself. This band is due to C=O stretching of an amide bond. From the structure of the resin, an amide bond formation is “key point” for the coupling CCTS with DHBA moiety. Accordingly, the additional bands appeared in the synthesized product showed the existence of DHBA moiety in the resin.

Figure 2 shows the results of acid-base titration for the synthesized CCTS-DHBA resin. From the structure of the resin, two pKa values of the resin can be expected, which are attributed to the hydroxyl groups of DHBA at *meta*- and *para*- positions. The pKa of 1,2-dihydroxy benzene are 9.34 and 12.60 [19], which can be referred to assign the pKa of hydroxyl groups in DHBA moiety. As shown in Fig.2, there are two pKa of 6.06 and 9.11 observed in the titration curve. The pKa of 9.11 may be attributed to the OH-group at *para*- position of the DHBA moiety, whereas the pKa of –OH group at *meta*- position could not be observed due to the limitation of titration method at high pKa of species (>12). The amino group of chitosan has a pKa of approx. 6.5 [20]. Therefore, the pKa of 6.06 in this resin is probably due to the amino group of chitosan, which means that some parts of amino group in CCTS did not react with DHBA moiety.

The first end point (A) is attributed to the neutralization of 3 mL of 0.10M HCl added to the resin solution in the initial step of titration. The result indicated that HCl was completely neutralized. The second endpoint (B) corresponds to neutralization of protonated amino group remaining in chitosan. From this result, it was found that about 0.60 mmol amino group of CCTS per gram resin did not react with DHBA moiety. The third end point (C) is attributed to the neutralization of protonated hydroxyl group at *para* position of DHBA moiety. From this result,

the amounts of DHBA moiety attached to cross-linked chitosan can be estimated to be 2.25 mmol g<sup>-1</sup> resin. Assuming that DHBA moiety is attached to all glucamine unit of chitosan, about 2.69 mmol DHBA may exist in 1 g of the resin. Therefore, the mole ratio of DHBA moiety to glucamine unit of chitosan can be estimated to be 0.84:1.00 (2.25 : 2.69); that is, 84% of amino group of CCTS is bound to DHBA moiety.

### ***3.2 Adsorption behavior of metal ions on the CCTS-DHBA resin***

The adsorption behavior of 60 elements on the CCTS-DHBA resin was examined by using the procedures given in section 2.4. Figure 3 shows the adsorption/recovery of 10 ng mL<sup>-1</sup> of the 60 elements in the pH range of 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-DHBA resin can adsorb vanadium, uranium, bismuth and gallium from acidic to alkaline pH region. Molybdenum could be adsorbed at around neutral pH regions, whereas copper, beryllium, nickel, aluminum, indium, lead, and silver are adsorbed on the resin from neutral to alkaline regions. Some elements, such as Al, Ga (pH  $\geq$ 5), Be (pH  $\geq$ 7), Cu (pH  $\geq$ 8), Pb (pH  $\geq$ 8) may retained on the resin without any interaction due to the formation of insoluble hydroxide species of the metals, whereas other elements or soluble species may adsorb on the resin by chelating or ion-exchange mechanism.

In the previous reports [15-16, 21-22], it was found that the cross-linked chitosan (CCTS) itself could adsorb aluminum, copper, molybdenum, vanadium, nickel, gallium, indium, bismuth and silver. Accordingly, the DHBA moiety attached to CCTS is seems to possess excellent

adsorption ability and selectivity for uranium, with less competitive to other elements at acidic regions. Then, pH 3 of the sample was selected for further experiments.

In general, uranium species varies over the pH range. Uranium becomes increasingly hydrolyzed and forms oligomeric species with the increasing pH. Many researcher reported [12, 23-24] that at  $\text{pH} \leq 4.3$ , uranium exist predominantly as monomeric species,  $\text{UO}_2^{2+}$ , and small amount as  $\text{UO}_2(\text{OH})^+$ . When the  $\text{pH} \geq 5$ , the formation of colloidal or oligomeric species, i.e  $(\text{UO}_2)_2(\text{OH})_2^{2+}$ ,  $(\text{UO}_2)_3(\text{OH})_5^+$ ,  $(\text{UO}_2)_4(\text{OH})_7^+$ ,  $(\text{UO}_2)_3(\text{OH})_7^-$ , are formed. Moreover, the precipitate of  $\text{UO}_2(\text{OH})_2$  may occurred. In this experiment, since pH 3 was selected for the collection/concentration of uranium in aqueous sample, the species of  $\text{UO}_2^{2+}$  predominate and are responsible for the adsorption of uranium on the CCTS-DHBA resin. Probably, uranium can adsorb on the resin with the chelating mechanism which is explained more detail in section 3.3.

### ***3.3 Adsorption capacity and chelate formation of uranium to CCTS-DHBA resin***

The adsorption capacity of the resin was examined by equilibrating 20 mg of CCTS-DHBA resin with a solution of uranium (VI) ion (100 mL,  $100 \mu\text{g mL}^{-1}$ ). The acidity of the solution was adjusted to pH 3. At appropriate time, 0.5 mL of the solution was taken and transferred to a clean bottle at certain time intervals until equilibrium was reached. Then, the concentration of uranium ion was measured by ICP- MS after appropriate dilution. The adsorption capacity of uranium was found to be  $330 \text{ mg g}^{-1}$  of resin, which is higher than other chelating resins reported so far (Table.1) as well as a commercially available resin for uranium, UTEVA ( $94.87 \text{ mg g}^{-1}$ ) [35]. In

addition, the loading half time ( $t_{1/2}$ ), defined as the amount of metal ion adsorbed on the resin is a half of its maximum adsorption capacity, was about 4 min, which provides fast sorption kinetics of the resin toward uranium.

Considering the quantities of the adsorbed uranium on the resin ( $330 \text{ mg g}^{-1} \text{ resin} \approx 1.39 \text{ mmol g}^{-1} \text{ resin}$ ) and the amount of DHBA moiety attached to the resin ( $2.25 \text{ mmol g}^{-1} \text{ resin}$ ), it can be predicted that uranium can form chelate of 1:2 (uranium to DHBA moiety) as illustrated in Fig. 4. The chelation of uranium as  $\text{UO}_2^{2+}$  occurs via the neighboring phenolic OH groups of DHBA moiety under the formation of a five-membered ring. Similar result was obtained when Alizarin (1,2-dihydroxy anthraquinone), which has phenolic OH groups in the same position as DHBA, was reacted with uranium. Stoichiometry of the chelate was found to be 1:2 (uranium to Alizarin)[36].

### ***3.4 Application of CCTS-DHBA resin to the collection/adsorption of uranium in water samples***

Table 2 shows the results obtained from the examination of the effect of cationic matrices, such as Na, K, Mg and Ca, on the recovery of uranium. The results indicate that the CCTS-DHBA resin is capable of adsorbing uranium quantitatively since the recoveries are almost complete (96.3-98.9 %) even in the presence of high cation matrices. Therefore, the CCTS-DHBA resin is very effective for the removal of the matrix components as the alkali and alkaline earth metal ions. Table 2 also shows the results of the recovery of uranium at various

concentrations in the sample solutions. In the wide concentration range ( $0.01 - 10 \text{ ng mL}^{-1}$ ), the CCTS-DHBA resin could adsorb uranium quantitatively with excellent recoveries (96.3-105%). The quantitative recoveries were also found after 20 cycles of column pretreatment procedures, which means that no loss of the activity and efficiency of the CCTS-DHBA resin after prolonged usage. This property is better than commercially available resin for uranium, UTEVA, in which the recovery of uranium decreased about 20% after 5 cycles [37].

To demonstrate the ability of the CCTS-DHBA resin for uranium collection, the preconcentration procedure using the CCTS-DHBA packed mini-column was also performed. Various concentration of uranium samples ( $1 - 100 \text{ pg mL}^{-1}$ , volume: 100 ml) were passed through the column and eluted with 10 mL of 1 M nitric acid. The results show that the recoveries of uranium were found in the range of 97.9 – 100.1% (Table 3), even when the alkali and alkaline earth matrices were added to the samples. These results indicate that the resin can be applied to column preconcentration/enrichment in high background matrix.

Table 4 shows the summary of the analytical results of uranium in tap water, river water, and seawater obtained by the proposed method with and without column enrichment. Because uranium in tap water sample exists at extremely low level ( $\text{pg mL}^{-1}$ ) and it close to LOD of ICP-MS ( $1 \text{ pg mL}^{-1}$ ), tap water sample in this work was concentrated by 10-fold. A quantitative amount of uranium was also spiked to water samples to ensure accuracy of the method. The recoveries were found to be good, indicating the suitability of CCTS-DHBA resin for the collection of uranium in various water samples.

The validation of the proposed method was tested by analyzing standard reference materials of river water (SLRS-4), nearshore seawater (CASS-4), and open ocean seawater (NASS-5) supplied by National Research Council, Canada. The samples were adjusted to pH 3 followed by procedure described in the experimental section. The results obtained are shown in Table 5, from which it is clear that the uranium concentration established by ICP-MS method in conjunction with CCTS-DHBA column pretreatment procedure agree well with the certified values.

#### **4. Conclusions**

The solid phase adsorbent, CCTS-DHBA resin, developed in this work by chemically bonding 3,4-dihydroxy benzoic acid (DHBA) moiety to amino group of cross-linked chitosan (CCTS) through amide bond formation, allows a rapid, precise, and relatively selective collection and enrichment of trace and ultra-trace amounts of uranium (pH 3) in tap water, river water and seawater samples. The collected uranium on the resin was easily eluted with 1M nitric acid. No loss of the activity and efficiency of the adsorption behavior after prolonged usage are the addition advantages of this resin. Moreover, the newly synthesized CCTS-DHBA resin offers high adsorption capacity for the uranium (VI) of  $330 \text{ mg g}^{-1}$  resin, which is better than other uranium resins reported so far.

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Table 1 Characteristics comparison of various resins for uranium

No	Solid support	Moiety	Capacity, mg g <sup>-1</sup>	Loading half time (t <sub>1/2</sub> ), min	pH range	Elution	Application	Ref.
	Cross-linked chitosan	Serine	249	-	3 - 9	1M HNO <sub>3</sub>	tap, river, and seawater	13
2	Amberlite XAD-16	[(2-hydroxyarsinoylphenylamino) ) methyl] phosphonic acid	59.5	4.6	5 - 6	1M (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	seawater and well-water	25
4	Amberlite XAD-4	succinic acid	12.3	0.5	4.5 - 8	1M HCl	soil, sediment	26
5	Amberlite XAD-4	bicine	90.4	5	4.5 - 9	1M HCl	synthetic sample	27
6	Amberlite XAD-4	o-vaniline semicarbazone	2.89	6.5	6.8 - 8	HNO <sub>3</sub> or HCl	geological sample	28
7	Amberlite XAD-4	5-aminoquinoline-8-ol	2.74	2.4	4 - 6	1M HCl	soil, sediment	29
8	Amberlite XAD-4	octacarboxymethyl-C- methylcalix[4]resorcinarene	64.3	-	5 - 7	0.4M HCl	synthetic sample	30
10	Naphthalene	5,7-dichloroquinoline-8-ol	1.88	-	4.5 - 7	-	soil, sediment	31
11	Benzophenone	1(2-pyridylazo)-2-naphthol	2.34	-	10.5 - 11	-	soil, sediment	32
12	Poly(styrene beta-hydroxylamine)	37,38,39,40,41,42-hexahydroxy 1,8,13,19,25,31-hexacarboxy calix[8] aren	97.82	12	4	2M HCl	monazite sand	33
13	Merrifield chloromethylated resin	11,23-disemicarbazono-26,28-n- dipropoxy-25,27-dihydroxy calix[4]arene	3.02	8.5	5.5 - 7	0.25M HCl	geological sample	34
14	Cross-linked chitosan	3,4-dihydroxy benzoic acid	330	4	3 - 9	1M HNO <sub>3</sub>	tap, river, and seawater	This work

Table 2 Recovery of uranium at various concentrations after pretreatment with CCTS-DHBA resin in the presence and absence of matrices

Concentration added <sup>*a</sup> / ng mL <sup>-1</sup>	Matrix	Found/ ng mL <sup>-1</sup>	Recovery (%)
0.01	none	0.01 ± 0.00	105
0.1	none	0.10 ± 0.00	98.5
1	none	0.99 ± 0.00	99.3
1	Artificial river water <sup>*b</sup>	0.98 ± 0.02	98.5
1	Artificial seawater <sup>*c</sup>	0.98 ± 0.03	98.1
10	none	9.80 ± 0.02	98.1
10	Artificial river water <sup>*b</sup>	9.63 ± 0.04	96.3
10	Artificial seawater <sup>*c</sup>	9.89 ± 0.09	98.9

5 <sup>a</sup> Samples (pH 3), 10 ml, were treated with the column (CCTS-DHBA resin: 1 mL; 0.2 g dry weight) followed by elution with 10 mL of 1M HNO<sub>3</sub> and measurement by ICP-MS

<sup>b</sup> Artificial river water matrices: Na (20 µg mL<sup>-1</sup>), K (10 µg mL<sup>-1</sup>), Mg(15 µg mL<sup>-1</sup>), Ca (50 µg mL<sup>-1</sup>).

10 <sup>c</sup> Artificial seawater matrices: Na(12000 µg mL<sup>-1</sup>), K (3900 µg mL<sup>-1</sup>), Mg (1200 µg mL<sup>-1</sup>), Ca (400 µg mL<sup>-1</sup>).

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Table 3 Recovery of uranium after preconcentration procedure using CCTS-DHBA resin

Concentration added <sup>*a</sup> / pg mL <sup>-1</sup>	Matrix	Enrichment factor	Found <sup>*b</sup> / pg mL <sup>-1</sup>	Recovery (%)
1	none	10	0.99 ± 0.01	99.5
10	none	10	9.90 ± 0.15	99.0
100	none	10	100.0 ± 1.1	100.1
100	Artificial river water <sup>*c</sup>	10	99.8 ± 1.6	99.8
100	Artificial seawater <sup>*d</sup>	10	97.9 ± 1.9	97.9

<sup>a</sup> Samples (pH 3), 100 ml, were treated with the column (CCTS-DHBA resin: 1 mL; 0.2 g dry weight) followed by elution with 10 mL of 1M HNO<sub>3</sub> and measurement by ICP-MS.

30 <sup>b</sup> Original concentration (concentrations measured were divided by the enrichment factor).

<sup>c</sup> Artificial river water matrices: Na (20 µg mL<sup>-1</sup>), K (10 µg mL<sup>-1</sup>), Mg(15 µg mL<sup>-1</sup>), Ca (50 µg mL<sup>-1</sup>)

35 <sup>d</sup> Artificial seawater matrices: Na(12000 µg mL<sup>-1</sup>), K (3900 µg mL<sup>-1</sup>), Mg (1200 µg mL<sup>-1</sup>), Ca (400 µg mL<sup>-1</sup>)

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Table 4 Analytical results of uranium in water samples after pretreatment with CCTS-DHBA resin

Sample	Added/ ng mL <sup>-1</sup>	Found/ ng mL <sup>-1</sup>	Recovery (%)
Tap water <sup>*a</sup>	0	0.0017 ± 0.0001	
River water (Asahi) <sup>*b</sup>	0	0.045 ± 0.002	
	0.05	0.096 ± 0.001	102
River water (Zasu) <sup>*b</sup>	0	0.044 ± 0.004	
	0.05	0.093 ± 0.002	98
Seawater (Shin Okayama Port) <sup>*c</sup>	0	2.23 ± 0.18	
	5	7.18 ± 0.12	99
Seawater (Shibukawa) <sup>*d</sup>	0	2.93 ± 0.13	
	5	7.74 ± 0.01	96

<sup>a</sup> Water sample (100 mL) was adjusted to pH 3, then passed through the CCTS-DHBA column (1 mL; 0.2 g dry weight) followed by elution with 10 mL of 1M HNO<sub>3</sub> and measurement by ICP-MS. Therefore, the sample was concentrated by 10-fold.

<sup>b</sup> Water samples (10 mL) were adjusted to pH 3, then passed through the CCTS-DHBA column (1 mL; 0.2 g dry weight) followed by elution with 10 mL of 1M HNO<sub>3</sub> and measurement by ICP-MS.

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Table 5 Analytical results of uranium in standard reference materials after pretreatment with CCTS-DHBA resin

Sample <sup>*a</sup>	Found/ ng mL <sup>-1</sup>	Certified/ ng mL <sup>-1</sup>
SLRS-4 (River water)	0.049 ± 0.003	0.050 ± 0.003
CASS-4 (Nearshore seawater)	3.09 ± 0.02	(3.0) <sup>*b</sup>
NASS-5 (Open Ocean seawater)	2.56 ± 0.13	(2.6) <sup>*b</sup>

80 <sup>a</sup> Samples (10 mL) were adjusted to pH 3, then passed through the CCTS-DHBA column (1 mL; 0.2 g dry weight) followed by elution with 10 mL of 1M HNO<sub>3</sub> and measurement by ICP-MS.

<sup>b</sup> Information only

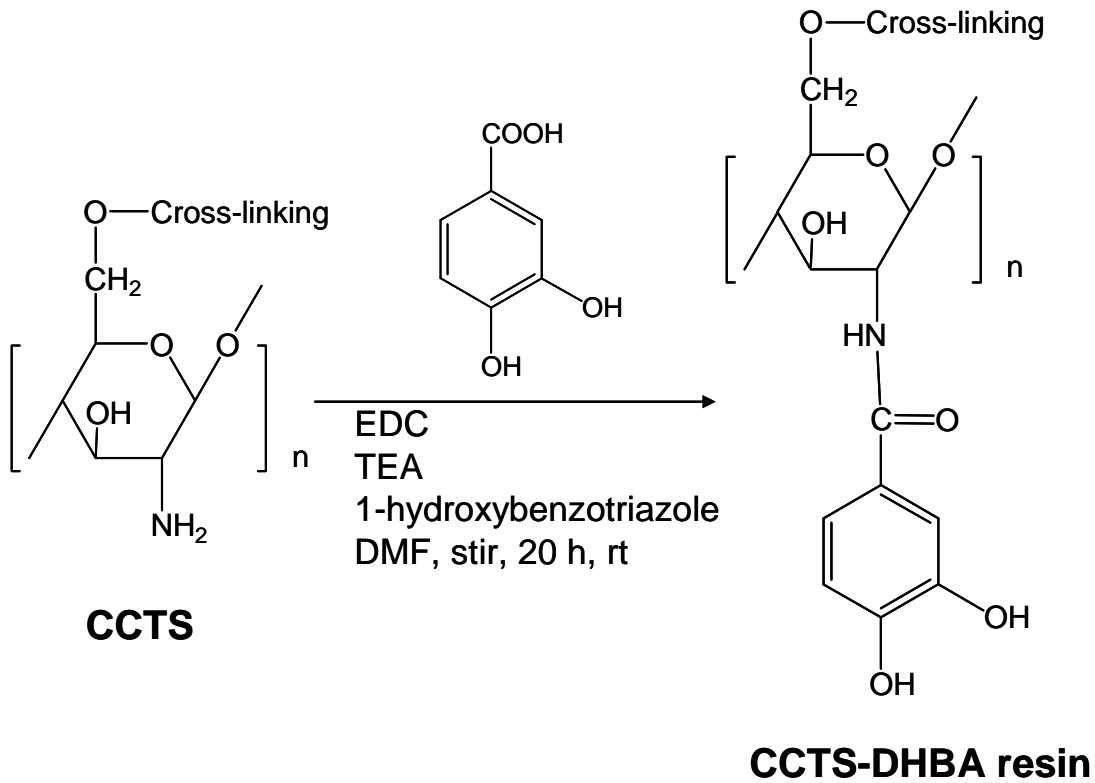


Fig. 1 Synthesis scheme of CCTS-DHBA resin

100 Cross-linking :  $\text{—CH}_2\text{—CH(OH)—CH}_2\text{—O—CH}_2\text{—CH}_2\text{—O—CH}_2\text{—CH(OH)—CH}_2\text{—}$

DHBA: 3,4-dihydroxy benzoic acid;

CCTS-DHBA : cross-linked chitosan possessing 3,4-dihydroxy benzoic acid moiety;

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

TEA : Triethylamine ; DMF : Dimethyl formamide.

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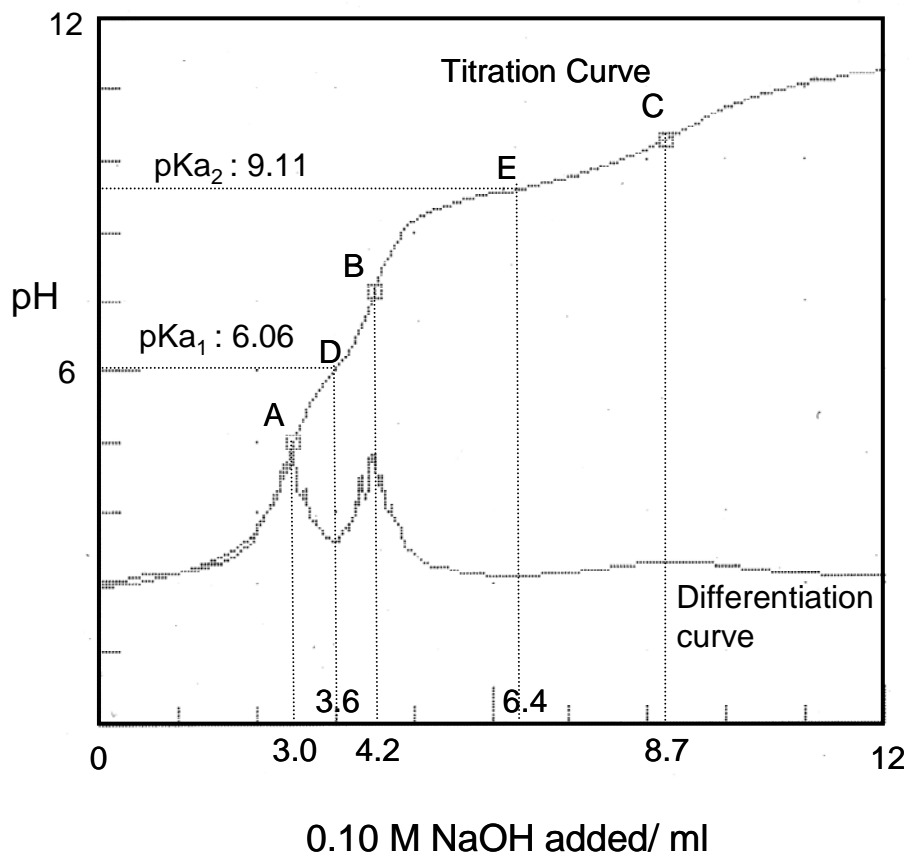


Fig. 2 Acid-base titration of the CCTS-DHBA resin.

115 A sample for the titration consists of 1 mL of the CCTS-DHBA resin (wet volume, 0.2 dry weight), 3 mL of 0.1 M hydrochloric acid and 27 mL of the ultrapure water;

A: the inflection point of hydrochloric acid; B, C: the inflection point of CCTS-DHBA resin;

D, E: the half point of the equivalent points.

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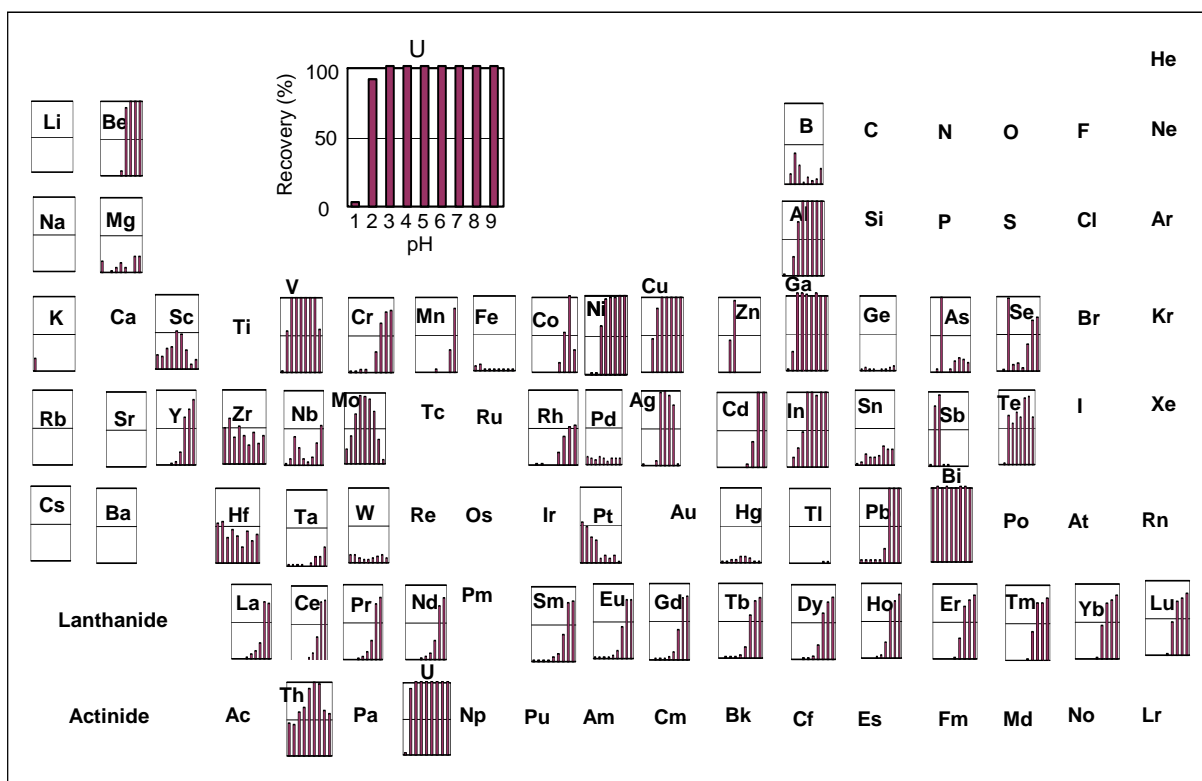


Fig. 3 Adsorption behavior of trace elements at various pHs with CCTS-DHBA resin.

Sample : 10 mL; concentration of each element in the samples: 10 ng mL<sup>-1</sup>; column: 1 mL

130 (0.2 g dry weight) of the CCTS-DHBA resin; all elements were measured by ICP-MS.

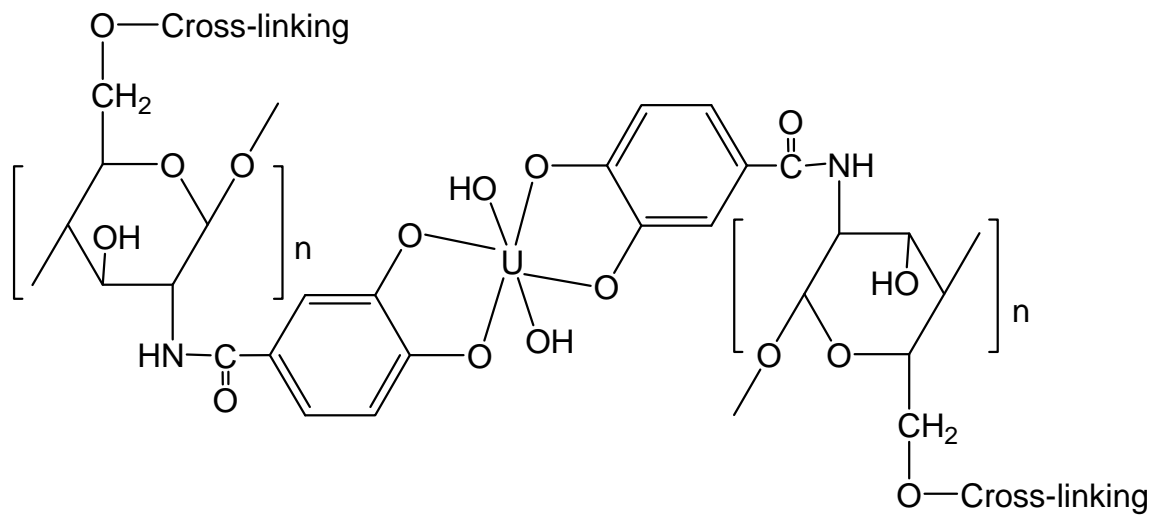


Fig 4 Predicted uranyl chelate in CCTS-DHBA resin