Synthesis of chitosan-based resins modified with tris(2-aminoethyl)amine moiety and its application to collection/concentration and determination of trace mercury by inductively coupled plasma atomic emission spectrometry

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Synthesis of Chitosan-based Resins Modified with Tris(2-aminoethyl)amine for Mercury Collection/Concentration and Determination

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A novel chitosan-based chelating resin modified with tris(2-aminoethyl)amine was synthesized, and the resin capability in the collection/concentration of mercury was examined. The synthesized resin showed good adsorption toward mercury in wide pH range, and the adsorbed mercury can be easily eluted by using 2 M HNO_3 without any addition of complexing agent. The resin was then packed in a mini-column and the mini-column was installed on an automated pretreatment system coupled with ICP-AES (Auto-Pret AES system). The proposed method had demonstrated that CCTS-TAA along with Auto-Pret AES system can be applied to the on-line concentration/collection and determination of mercury in aqueous solution.

1 Introduction

Mercury (Hg) is one of the most toxic heavy metals commonly found in the global environment layer [1]. Mercury represents a serious environmental problem because it is widely used in many industries and applications [2]. The toxicity of mercury even at very low levels has led to its stringent control with a maximum contaminant level of 2 $\mu g\ L^{-1}$ set by the US Environmental Protection Agency [3]. This regulated value is even lower in Japan, as it was set to 0.5 $\mu g\ L^{-1}$ by Japan ministry of environment [4]. Since the regulated value and concentration of mercury in natural waters are very low, hence, powerful techniques to monitor Hg in natural water are required. In order to achieve accurate, reliable and sensitive results, the separation and preconcentrations step is necessary prior to analysis.

In recent year, solid phase extraction has received considerable interest for the enrichment of metal ions for their accurate determination at trace levels. The importance of separation and concentration technique involving chelating sorbent in trace analysis has risen substantially because it can increase analyte concentration to a detectable level, while at the same time can eliminate matrix effects [5].

Mercury ion interacts strongly with ligands containing nitrogen and sulfur donor atoms [6]. Several sorbent containing nitrogen and/or sulfur ligands have been proposed for mercury enrichment [7-11].

Recently, much attention has been paid to the adsorption of metal ions on various kinds of biomass. Among those biomass, chitosan has been proved to be an extremely promising material due to its high chelating ability, higher hydrophilicity, environmentally safe, and abundant base material. Chitosan also has been proposed as biopolymers for mercury removal [12-14]. However, the application of chitosan to Hg determination is less reported.

In this work, the crosslinked chitosan functionalized tris(2-aminoethyl)amine (CCTS-TAA) moiety was synthesized to improve chitosan capability and selectivity for Hg adsorption from aqueous solution. The potential of the synthesized resin to be applied to Hg determination was also demonstrated.

2 Experimental

2.1 Instruments

An inductively coupled plasma mass spectrometer (ICP-MS) SPQ8000H System Seiko Instruments (Chiba, Japan) was used for investigating the adsorption behavior of various elements on CCTS-tris(2-aminoethyl)amine resin. Infrared spectra (4000–400 cm^{-1}) were recorded by using Jasco FT/IR-4100 Jasco International Co. Ltd. (Tokyo, Japan) for characterization of the synthesized-resin. ICP-AES Vista-pro Seiko Instruments (Chiba, Japan) was coupled with Auto-Pret system to investigate the possibility of resin application to the determination of mercury in aqueous solution.

2.2 Reagents

Chitosan, flake type with 80% deacetylation degree, and tris(2-aminoethyl)amine were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). All other reagents used for the synthesis of CCTS-Tris(2-aminoethyl)amine resin were of analytical reagent grade.

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

Ultrapure grade nitric acid (60%, density 1.38 $\mathrm{g\ mL}^{-1}$) Kanto Chemicals (Tokyo, Japan) was diluted with ultrapure water to give 1 M nitric acid. Acetic acid (minimum 96%) and ammonia water (29%), which were used for preparing ammonium acetate buffer solutions, were electronic industrial reagent grade Kanto Chemicals（Tokyo, Japan). Ultrapure water

(18.3 MΩ cm^{-1} resistance) prepared by Elix 3/Milli-Q Element system Nihon Millipore (Tokyo, Japan) was used for diluting standard solution.

2.3 Synthesis of CCTS-TAA

The cross-linked chitosan modified with tris(2-aminoethyl)amine was synthesized in two major steps: synthesis of crosslinked chitosan and introduction of tris(2-aminoethyl)amine moiety.

Chitosan dissolves in acidic medium which is considered to be a serious disadvantage from the view-point of metal analysis. Therefore, in order to improve the mechanical and chemical durability of chitosan, a cross-link structure of chitosan was synthesized with ethyleneglycoldiglycidylether (EGDE) as the cross-linker. Among two reactive groups in chitosan, hydroxyl group was used as a terminal for the cross-link structure, while the reactive amino group was kept for further derivatization. Chitosan's durability was improved by synthesizing a cross-link structure of chitosan with ethyleneglycoldiglycidylether (EGDE) as the cross-linker. The detail of synthesis procedure of EGDE cross-linked chitosan had been reported by other co-workers [13].

In the second step, cross-linked chitosan (5 g) was reacted with epibromohydrin (30 g) in order to attach an extension arm of epibromohydrin, giving cross-linked chitosan (CCTS) epibromohydrin. This extension arm provided bromo group which serves as leaving group and facilitated the introduction of the moiety. The product was then mixed with tris(2-aminoethyl)amine (10 g) in dioxane (100 mL), and the mixture was refluxed for 3-h. In gaseous phase, tertiary amine possesses higher nucleophilicity compared to primary amine because the central nitrogen atom was surrounded by alkyl group which act as electron donating group. However, in aqueous solution, the stabilization role is mainly played by solvation which involves hydrogen bonding. In this situation, primary amine has a higher nucleophilicity because it can form hydrogen bonding by the presence of N-H which is not possessed by tertiary amine, thus the coupling was likely to take place through primary amine group. The complete synthesis scheme of CCTS-TAA is shown in Fig. 1.

2.4 On-line metal collection and determination procedure

The manifold of Auto-Pret AES system is shown in Fig. 2. CCTS-TAA resin was packed in a PTFE column (2 mm i.d x 4 cm), and the column was installed on SWV in the position as shown in Fig. 2. The on-line pretreatment procedure was carried out in four major steps: *column conditioning*, *preconcentration*, *washing*, and *elution*.

Column conditioning step: 1 mL of 0.5 M ammonium acetate solution was aspirated at flow rate of 400 μL s^{-1} into holding coil through the port 3 of SLV. While the SWV was in the load position, the solution in the holding coil was injected at flow rate of 40 μL s^{-1} into the column in order to adjust the pH of the resin.

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

Washing step: 1 mL of ultrapure water was aspirated at flow rate of 400 μL s^{-1} into the syringe pump, and then the water was injected into the column at flow rate of 40 μL s^{-1}. This process will force the sample remained in the line to pass through the column, and at the same time remove matrices and the un-adsorbed metal ions.

Elution step: 0.5 mL of 2 M nitric acid was aspirated into the holding coil at flow rate of 400 μL s^{-1} from the port 5 of SLV, and. The position of syringe pump was set to 2.5 mL by aspirating ultrapure water into the syringe pump. The position of SWV was turned to inject-position, and the eluent was flowed into the column at flow rate of 30 μL s^{-1}, and the effluent zone moved to the ICP-AES for the element measurement.

2.5 On-line metal collection for adsorption behavior study

The adsorption behavior of CCTS-TAA resin was investigated by using on-line mini column procedure where CCTS-tris(2-aminoethyl)amine resin was packed in a small PTFE column (2 mm i.d. × 40 mm), and the column was installed on Auto-pret System. The procedure for carrying out this work is similar with the procedure which had been described in section 2.4 above, except 2.5 mL of sample and 2.5 mL of eluent was used, and the effluent obtained from this procedure was transferred into a polypropylene test tube and then analyzed with ICP-MS.

3 Results and Discussion

3.1 Characteristics of CCTS-TAA

The products from each synthesis step were characterized by measuring their IR spectra. The peak at 894 cm^{-1} in the spectrum of cross-linked chitosan confirms the N–H wagging of the amino group. This band intensity was decreased when the extension arm of epibromohydrin was attached through the amino group. In CCTS-epibromohydrin, the peak C-Br can be clearly observed in 500-600 cm^{-1}. The intensity of this peak was decreased in the spectra of final product as expected, since the reaction involve the substitution of Bromo group by tris(2-aminoethyl)amine.

3.2 Adsorption behavior of Hg on CCTS-TAA

The adsorption behavior study had shown that CCTS-tris(2-aminoethyl)amine has a good affinity toward Hg as can be expected from the presence of amine group. It is known that N and S ligands possess affinity to adsorb Hg^{2+} which belongs to group III “soft” cation. Although, S ligand possess better affinity to bind soft cation compared to N, but usually the retention of soft cation in S ligand is strong thus a complexing agent is necessary for elution. The CCTS-tris(2-aminoethyl)amine possesses tertiary amine and primary amine which serve as ligand for Hg complexation. Compared to primary amine, tertiary amine has a higher affinity and selectivity for Hg(II) from aqueous solution [15]. The proposed chelation structure of Hg by CCTS-TAA is shown in Fig. 3**Error! Reference source not found.**.

The adsorption of Hg on CCTS-tris(2-aminoethyl)amine can be carried out at pH ≥ 3 with excellent recovery value when 2 M nitric acid was used as the eluent. Although CCTS itself had been reported to have capability in mercury adsorption, the adsorption only takes place when sample was prepared in HCl solution [13]. In this case, Hg forms stable complex with Cl, and the adsorption occurs between $HgCl_2$ and primary amine through coordination with lone pair in nitrogen atom. Moreover, the Hg adsorbed on CCTS could not be removed by simply using nitric acid and thus the addition of thiourea into the eluent was necessary [13]. While in CCTS-TAA, it was demonstrated that the elution of adsorbed Hg can be easily carried out by using 2 M nitric acid without addition of complexing agent. The resin cannot adsorb Hg at pH lower than 3, which supported that Hg could be easily desorbed by using strong acid.

The overall result of CCTS-TAA adsorption behavior study is summarized in Fig. 4, expressed as the recovery of each 10 $\mu g\ L^{-1}$ of 57 elements when the adsorption was carried out in various pH.

3.3 On-line collection and determination of Hg with CCTS-TAA and Auto-Pret AES system

The possibility for applying CCTS-TAA to Hg collection and determination using Auto-pret System was investigated. The result showed that a good peak profile was observed when a solution containing 5 ng mL^{-1} Hg was injected as a sample, as shown in Fig 5. The trailing signal which appeared next to the peak was a result from the common memory effect of Hg on ICP-AES. However, the signal rapidly went down to baseline during the process of preconcentration of the next sample, and thus the next measurement sequence should not be affected by such drawback.

4. Conclusion

A novel chitosan resin functionalized with tris(2-aminoethyl)amine moiety was synthesized and its capability to adsorb Hg at various pH was examined. The synthesized resin showed good adsorption toward Hg at $pH \geq 3$ with excellent recovery value when 2 M of HNO_3 was used as the eluent. Compared to Hg adsorption on CCTS, the adsorbed Hg on CCTS-TAA can be easily eluted by using HNO_3 without any addition of complexing agent. The method has demonstrated that CCTS-TAA which was packed in a mini column and then installed on with Auto-Pret AES system can be applied to the on-line determination of Hg.

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List of Figures

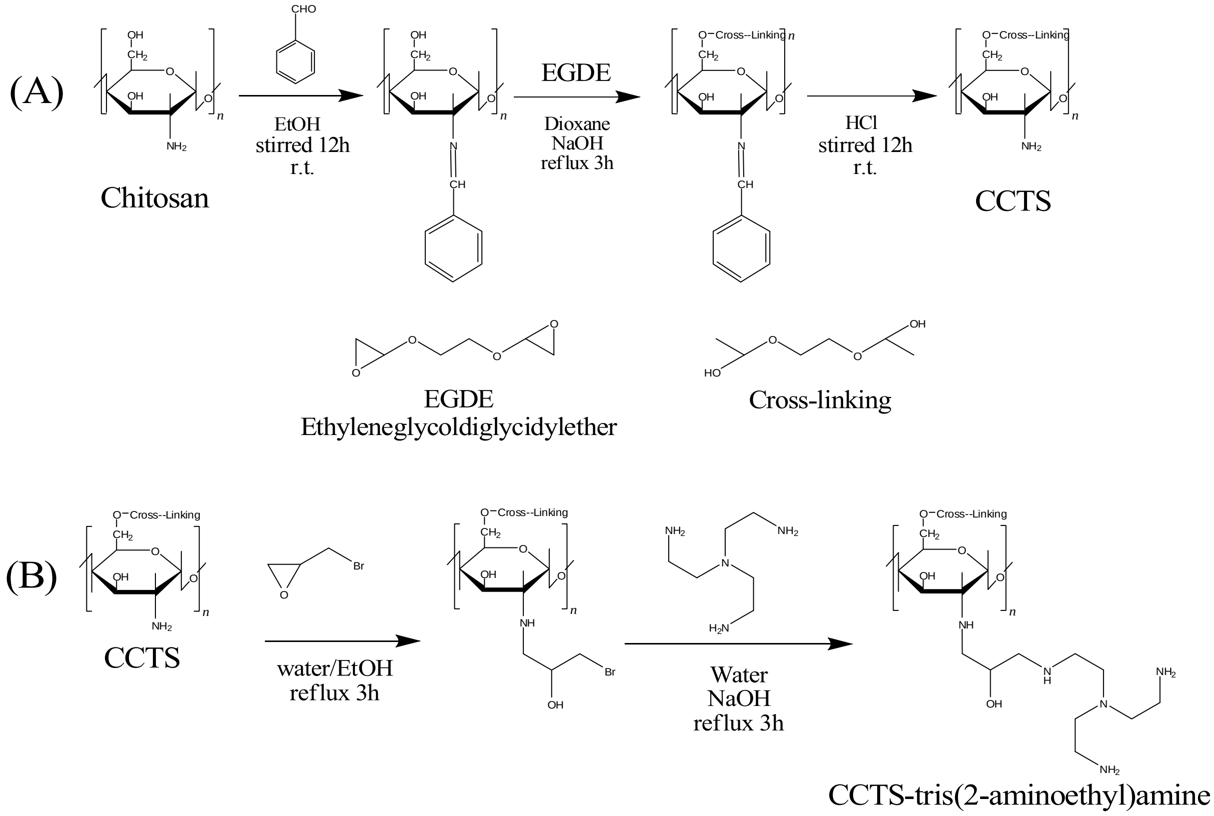

Fig. 1. *Synthesis Scheme of CCTS-TAA*

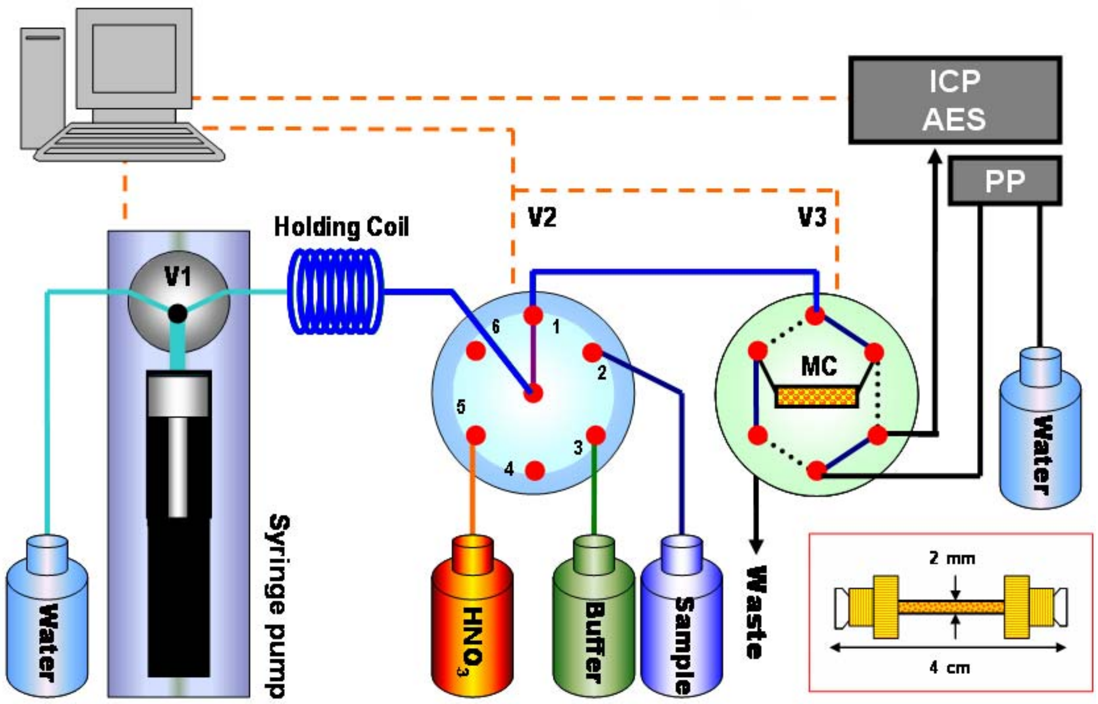

Fig. 2. *Manifold of Auto-Pret AES System. MC: mini column; PP: ICP-AES peristaltic pump; V1: syringe valve; V2: 6-ports selection valve; V3: six ways switching valve.*

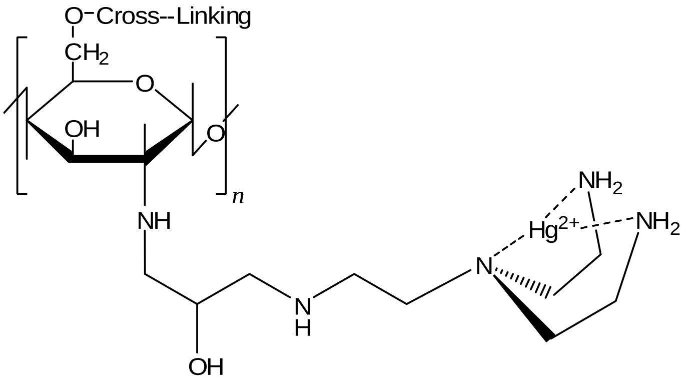

Fig. 3 *Proposed structure of Hg chelation on CCTS-TAA*

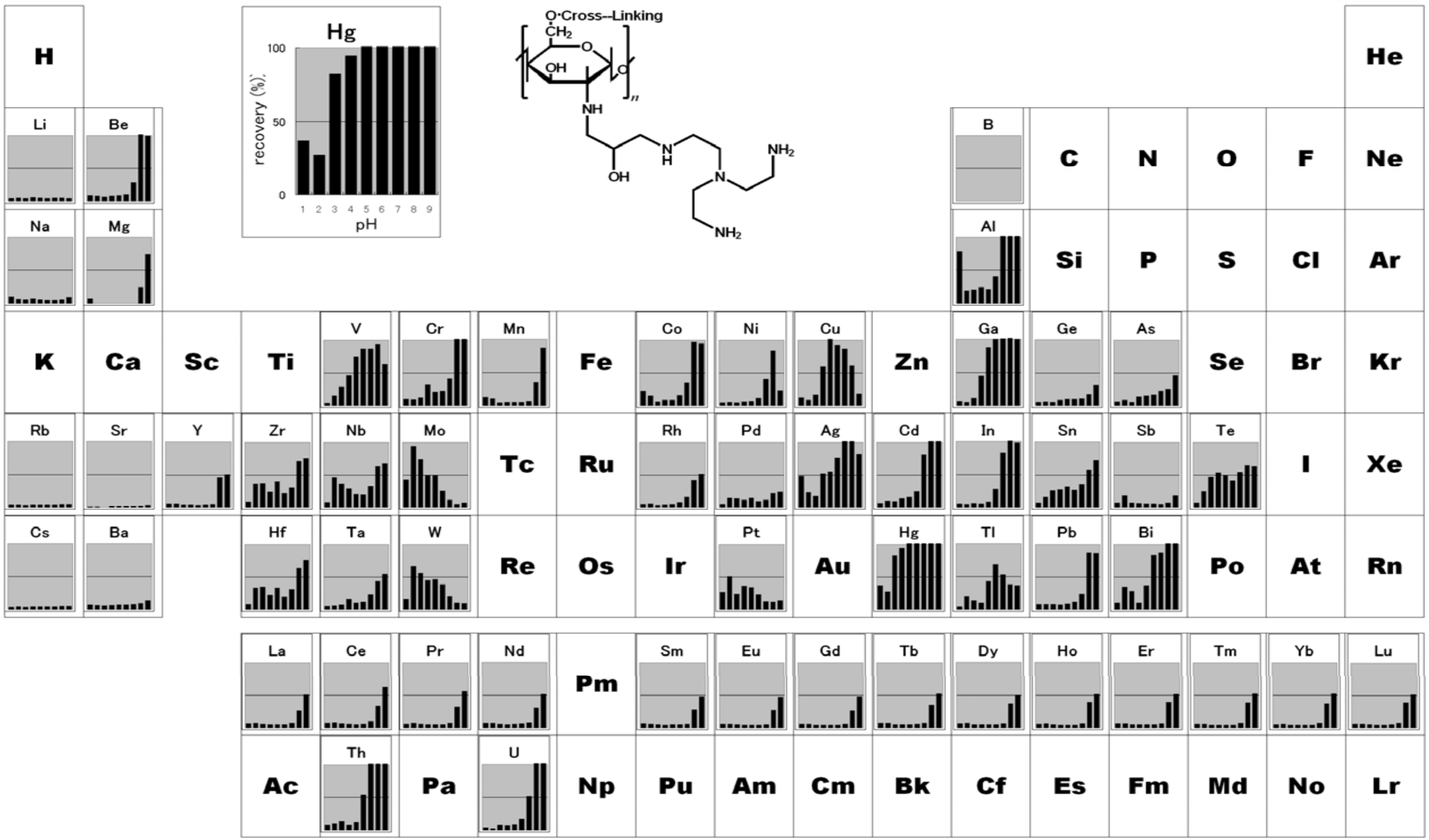

Fig. 4 *Adsorption of various elements on CCTS-tris(2-aminoethyl)amine.*

Sample: 2.5 mL of solution containing 10 ppb various elements; elution: 2.5 mL of 2 M HNO_3*;*

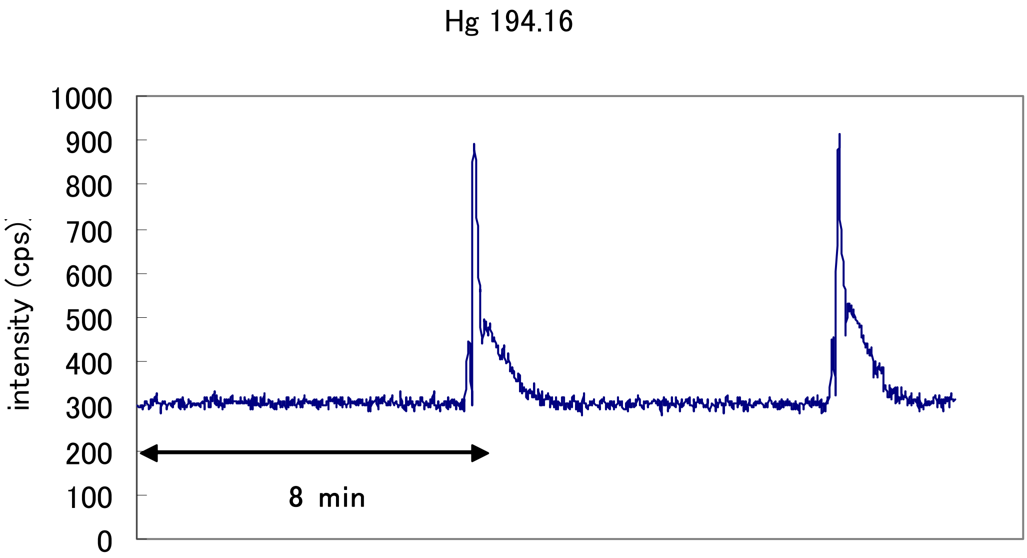

Fig. 5 *Peak profile of 5 ng mL^{-1} Hg obtained by using Auto-Pret AES System with CCTS-TAA. Sample: 5 mL; Eluent: 0.5 mL of 2 M HNO$_3$; Sample flow rate: 30 μL s^{-1}; Eluent flow rate: 30 μL s^{-1}*