



Short communication

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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ABSTRACT

A novel chitosan-based chelating resin modified with tris(2-aminoethyl)amine moiety (CCTS-TAA) was synthesized, and its characteristics in the collection/concentration of mercury was examined. The synthesized resin showed good adsorption toward mercury in a wide pH range, and the adsorbed mercury can be easily eluted by using 2 M HNO₃ without any addition of complexing agent. The resin was then packed in a mini-column and the mini-column was installed on a computer-controlled automated-pretreatment (Auto-Pret) system coupled with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) for on-line mercury collection and determination at trace level.

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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 to produce various kinds of products [2]. The toxicity of mercury even at very low levels has led to its stringent control with a maximum contaminant level of 2 ng mL⁻¹, which has been set by the US Environmental Protection Agency [3]. This regulated value is even lower in Japan, as it was set to 0.5 ng mL⁻¹ by Japan Ministry of Environment [4]. The regulated value and the concentration of mercury in natural waters are very low; hence, powerful techniques to monitor mercury in natural water are required. In order to achieve accurate, reliable and sensitive results, the separation and preconcentration step for mercury is necessary prior to its measurement.

In recent years, solid phase extraction (SPE) method 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 it can eliminate matrix effects [5].

In general, mercury ion interacts strongly with ligands containing nitrogen and sulfur atoms [6]. Several sorbent containing nitrogen and/or sulfur ligands have been proposed for mercury enrichment [7–11]. Tris(3-aminoethyl)amine, which possesses several amine groups, was expected to have a potential to form chelate with mercury ion.

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 has been hardly reported.

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

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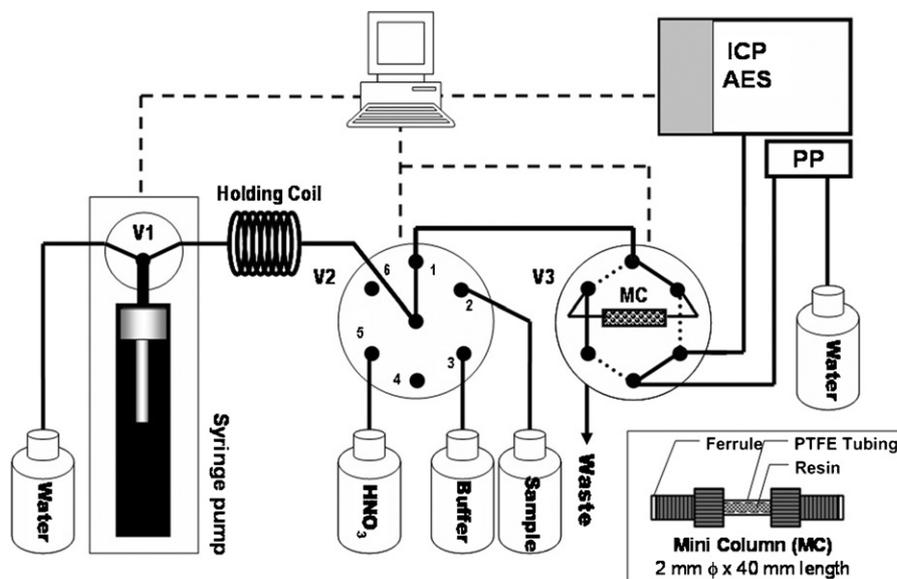


Fig. 1. Manifold of Auto-Pret AES System. MC: mini-column; PP: ICP-AES peristaltic pump; V1: syringe valve; V2: 6-ports selection valve; V3: 6-way switching valve. Solid-line shows the load (pretreatment) position, and dotted-line shows inject (elution) position.

on-line preconcentration procedure, where less contamination, less reagent consumption, and less waste-generation can be achieved [15–21].

In this work, the cross-linked chitosan (CCTS) functionalized with tris(2-aminoethyl)amine (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. This work was a fundamental study and dedicated to explore the potential of the synthesized resin to concentrate inorganic Hg(II) as well as the possibility of this resin to be utilized in an automated-pretreatment system (Auto-Pret) prior to its application to determine mercury in environmental samples.

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-TAA. Infrared spectra ($4000\text{--}400\text{ cm}^{-1}$) were recorded by using Jasco FT/IR-4100 Jasco International Co. Ltd. (Tokyo, Japan) for characterization of the synthesized-resin. A modular digital syringe pump Cavro (San Jose, CA, USA), a selection valve and a switching valve Hamilton (Reno, NV, USA) were used as a part of Auto-Pret system. This Auto-Pret system was coupled with ICP-AES Vista-pro Seiko Instruments (Chiba, Japan) to demonstrate the potential of the synthesized resin to be applied 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-TAA were of analytical reagent grade.

Multi-element standard solutions were prepared from several kinds of single element standard solution for atomic absorption ($1000\text{ }\mu\text{g mL}^{-1}$) purchased from Wako Pure Chemicals (Osaka,

Japan). Two multi-element stock solutions for ICP-MS, XSTC-13 and XSTC-1, provided by Spex CertiPrep Inc. Metuchen (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.

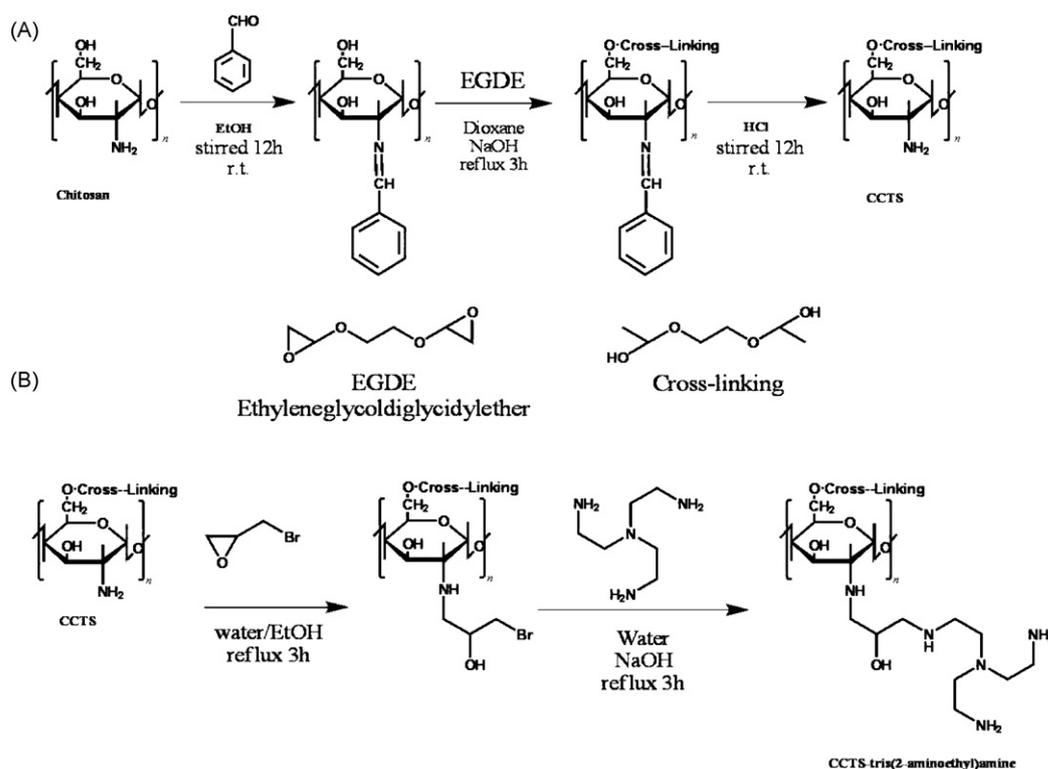
Ultrapure grade nitric acid (60% , density 1.38 g mL^{-1}) purchased from Kanto Chemicals (Tokyo, Japan) was diluted with ultrapure water to give 1 M nitric acid. Acetic acid (minimum 96%) and ammonia water (29%), which were used for preparing ammonium acetate solutions, were electronic industrial reagent grade purchased from Kanto Chemicals (Tokyo, Japan). Ultrapure water ($18.3\text{ M}\Omega\text{ cm}^{-1}$ resistance) prepared by Elix 3/Milli-Q Element system Nihon Millipore (Tokyo, Japan) was used for diluting standard solution.

2.3. Synthesis of CCTS-TAA resin

The CCTS-TAA resin was synthesized in two major steps (Scheme 1): (1) The synthesis of cross-linked chitosan, and (2) introduction of tris(2-aminoethyl)amine moiety into cross-linked chitosan.

Chitosan dissolves in acidic medium and this characteristic is considered to be a serious disadvantage from the view-point of metal collection based on solid phase extraction method. Therefore, in order to improve the mechanical and chemical durability of chitosan in acidic medium, a cross-linked structure of chitosan was synthesized with ethyleneglycoldiglycidylether (EGDE) as the cross-linker. Among two reactive groups in chitosan, the hydroxyl group was used as a terminal for the cross-link structure, while the reactive amino group was preserved for further derivatization. The detail of synthesis procedure of EGDE cross-linked chitosan had been reported by Motomizu and co-workers [13].

In the second step, EGDE cross-linked chitosan (5 g) was reacted with epibromohydrin (30 g) in order to introduce an extension arm of epibromohydrin into CCTS, giving CCTS-epibromohydrin. With bromo functional-group on its terminal which serve as a leaving group, this extension arm can facilitate the introduction of the tris(2-aminoethyl)amine moiety. The CCTS-epibromohydrin was then mixed with TAA (10 g) in dioxane (100 mL), and the mixture



Scheme 1. Synthesis of CCTS-TAA.

was refluxed for 3-h. Tertiary amine possesses higher nucleophilicity compared to primary amine because the central nitrogen atom was surrounded by alkyl group which act as an electron donating group. However, in aqueous solution, the stabilization role is mainly played by the solvation which involves hydrogen bonding. In this situation, primary amine has a higher nucleophilicity because it can form hydrogen bonding with the presence of N–H, thus the coupling was likely to take place through primary amine group.

2.4. On-line metal collection and determination procedure

The manifold of Auto-Pret AES system for on-line Hg collection/concentration and determination is shown in Fig. 1. CCTS-TAA resin was packed in a polytetrafluoroethylene (PTFE) column (2 mm i.d. \times 4 cm), and the column was installed on switching valve (SWV) at the position shown in Fig. 1. PTFE tubing with 0.8 mm of inner diameter was used for all connecting lines, except for holding coil where PTFE tubing with 2 mm of inner diameter was used. The on-line pretreatment procedure was carried out in four major steps: column conditioning, preconcentration, washing, and elution.

2.4.1. Column conditioning step

One milliliter of 0.5 M ammonium acetate solution of appropriate pH was aspirated at flow rate of $400 \mu\text{L s}^{-1}$ into holding coil through the port 3 of selection valve (SLV). While the SWV was kept in load position, the solution in the holding coil was propelled at flow rate of $40 \mu\text{L s}^{-1}$ into the column in order to adjust the pH of the resin.

2.4.2. Preconcentration step

Five milliliters of sample was aspirated at flow rate of $400 \mu\text{L s}^{-1}$ into the holding coil through the port 2 of SLV. The aspirated sample was then propelled into the column at flow rate of $30 \mu\text{L s}^{-1}$ for the metal collection on the resin and the removal of the matrices.

2.4.3. Washing step

One milliliter of ultrapure water was aspirated at flow rate of $400 \mu\text{L s}^{-1}$ into the syringe pump, and then the ultrapure water was propelled into the column at flow rate of $40 \mu\text{L s}^{-1}$. This process will force the remaining sample in the connecting-line to pass through the column, and at the same time this process will remove matrices and the un-adsorbed elements.

2.4.4. Elution step

0.5 mL of 2 M nitric acid was aspirated into the holding coil at flow rate of $400 \mu\text{L s}^{-1}$ from the port 5 of SLV, and then 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 propelled into the column at flow rate of $30 \mu\text{L s}^{-1}$; the effluent zone moved to the ICP-AES for the metal measurement.

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

2.5. On-line metal collection procedure for CCTS-TAA adsorption behavior study

The adsorption behavior of CCTS-TAA resin was investigated by using on-line mini-column procedure, where CCTS-TAA was packed in a small PTFE column (2 mm i.d. \times 40 mm) and the column was installed on Auto-Pret AES System. The on-line procedure of this work is similar to that described in Section 2.4, except for 2.5 mL of sample was used in preconcentration step, 2.5 mL of eluent was used in elution step, and the effluent obtained from this procedure was transferred into a polypropylene test tube and then analyzed with ICP-MS.

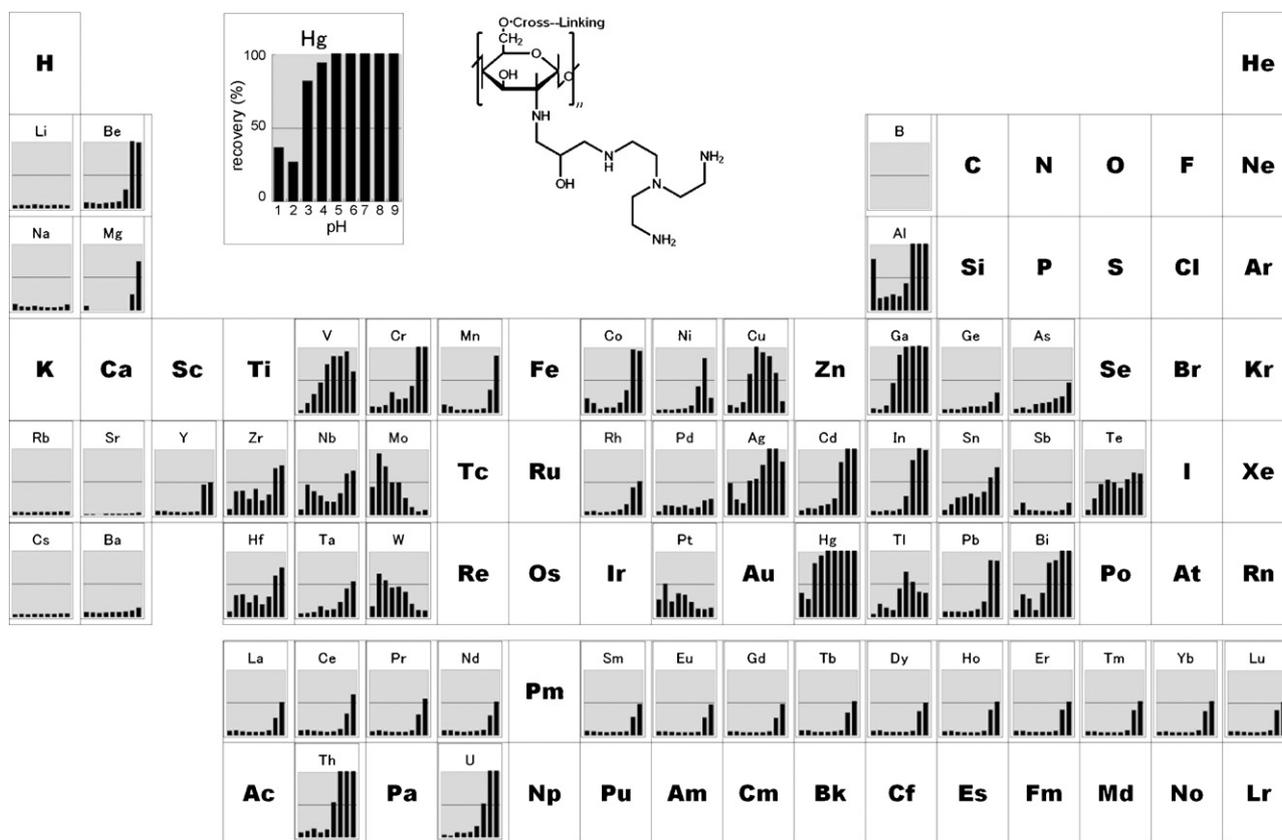


Fig. 2. Adsorption of various elements on CCTS-TAA. Sample: 2.5 mL of solution containing 10 ppb of various elements; Elution: 2.5 mL of 2 M HNO_3 .

3. Results and discussion

3.1. Characteristics of CCTS-TAA

The products obtained 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 $500\text{--}600\text{ cm}^{-1}$. The intensity of this peak was decreased in the spectra of final product which indicate that the substitution of bromo group by tris(2-aminoethyl)amine was occurred in the reaction.

3.2. Adsorption of metal ions on CCTS-TAA

The result of CCTS-TAA adsorption behavior study is summarized in Fig. 2, expressed as the recovery of each $10\text{ }\mu\text{g L}^{-1}$ of 57 elements when the adsorption was carried out in various pH.

CCTS-TAA has a good affinity toward Cr, Co, Cu, Ag, Al, Ga, In, Bi, and Hg, of which Co, Cu, Ag, and Hg can be expected due to the presence of amine group. The affinity toward Cr, Al, Ga, In, and Bi is extraordinary from the view-point of chelating mechanism. One possibility for such affinity is considered from the ion exchange mechanism, in which anionic species can be trapped on the positively charged amino groups: Cr species, as well as Al, Ga, In, and Bi are possibly present as an anionic species in alkaline solution.

It is well 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, usually the retention of soft cation on S ligand is so strong that a com-

plexing agent is necessary for elution [22]. The CCTS-TAA possesses tertiary amine and primary amine which can serve as a ligand for Hg complexation under moderate affinity. Compared to primary amine, tertiary amine has a higher affinity and selectivity for Hg(II) in aqueous solution [6]. The proposed chelation structure of Hg by CCTS-TAA is shown in Fig. 3.

The adsorption of Hg on CCTS-TAA can be carried out in wide pH range ($\text{pH} \geq 3$) with excellent recovery value when 2 M nitric acid was used as the eluent. Although CCTS itself was reported to have a good adsorption toward mercury, the adsorption only takes place when sample was prepared in HCl solution [13]. In that case, Hg forms stable complex with Cl^- , and the adsorption occurs through ion exchange mechanism. Moreover, the adsorbed Hg on CCTS could not be removed with nitric acid, and thus the addition of thiourea into the eluent was necessary [13]. Another work on mercury preconcentration by using YPA_4 chelating resin

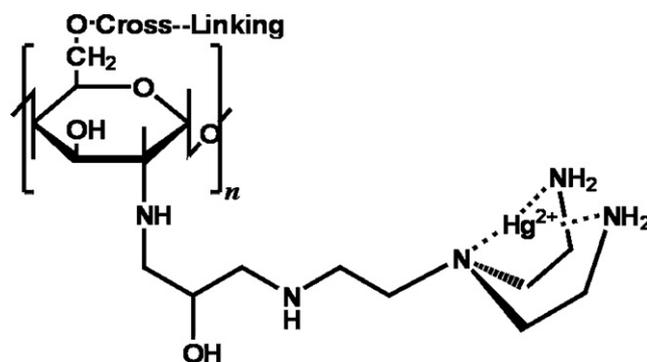


Fig. 3. Proposed structure of Hg chelation on CCTS-TAA.

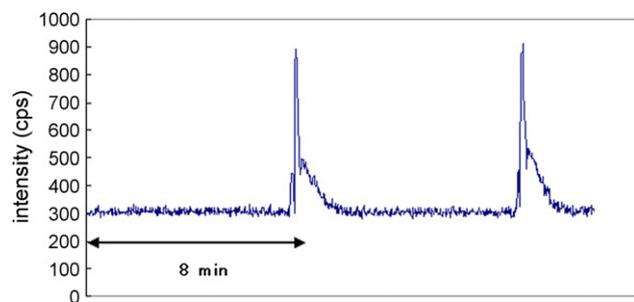


Fig. 4. Peak profile of 5 ng mL^{-1} Hg obtained by using Auto-Pret AES system with CCTS-TAA. Sample: 5 mL; Eluent: 0.5 mL of 2 M HNO_3 ; Sample flow rate: $30 \mu\text{L s}^{-1}$; Eluent flow rate: $30 \mu\text{L s}^{-1}$.

was also reported to require thiourea addition into the eluent [23]. In CCTS-TAA, it was found that the elution of adsorbed Hg can be easily carried out by using 2 M nitric acid without any addition of complexing agent. The resin cannot adsorb Hg at pH lower than 3, which indicates that Hg could be easily desorbed by using strong acid.

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

The possibility for applying CCTS-TAA to Hg collection/concentration and determination using Auto-Pret AES system was investigated. The result showed that a good peak profile was observed when a solution containing 5 ng mL^{-1} Hg was used as a sample, as shown in Fig. 4. The tailing signal, which appeared after the sharp peak, was a result from the common memory effect of Hg on ICP-AES. However, the signal reproducibility is enough for reproducible measurement of Hg and 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.

The limit of detection (LOD) corresponding to $S/N=3$ for Hg measurement was found to be 0.1 ng mL^{-1} for 5 mL of sample, and lower LOD can be expected when larger volume of sample is used. This LOD is lower compared to another work which utilize Hg-imprinted thiol functional sorbent with the same detector [8]. Another work on sequential cloud-point extraction method by using the same detector was reported to have lower LOD, but the extraction procedure was tend to be laborious and more time-consuming [24,25].

4. Conclusion

A novel chitosan resin functionalized with tris(2-aminoethyl) amine moiety was synthesized, and its ability to adsorb Hg at various pH was examined. The synthesized resin showed good adsorption toward Hg in wide pH range ($\text{pH} \geq 3$) 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 proposed method has showed that CCTS-TAA which was packed in a mini-column and then installed on Auto-Pret AES system can be applied to the on-line collection/concentration and determination of trace amounts of Hg.

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