

Separation and preconcentration of trace indium (III) in human urine by a commercially available iminodiacetate resin

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Abstract—Aim: The aim of this paper is to describe a simple and reliable method for the selective preconcentration and trace determination of indium in human urine.

Method: A solid phase extraction procedure for graphite furnace atomic absorption spectrometric determination of In(III) is provided on Chelex-100 resin. The sample solutions at pH 6.5 were pumped through a micro-column at the rate of 1.0 mL/min by using a peristaltic pump. The elution of the retained analytes on the column was performed with 1.5 mL of 0.1 M HNO₃ at 1.0 mL/min flow rate.

Results: The proposed procedure is successfully applied to determine the concentrations of indium ion in urine samples with linear correlation coefficient (>0.9992) and linear range 20-250 µg/L. The limit of detection of the proposed method is 2.75 µg/L. The optimum analytical procedure was applied to assay of real urine and standard reference material, with good recoveries of 92.6-103.3% (n=6) and good reproducibility (RSD < 4.9%).

Keywords: Indium; urine; preconcentration; chelex-100 resins

I. INTRODUCTION

Indium (In) is a trace and toxic element and of concern due to their widespread medical and radiodiagnostic applications. It has been shown that exposure to indium compounds induces genotoxicity or carcinogenicity [1]. The determination of indium in biological samples (urine, blood, and scalp hair) of human beings has attracted considerable interest [2]. However, the direct determination of extremely low concentrations of indium in urine is limited by not only low sensitivity, but also by matrix interference. For this reason, the separation and preconcentration of trace indium from urine matrix is often required. The most widely used techniques include liquid-liquid extraction (LLE) [3], ion-exchange [4] and solid-liquid separation [5, 6] and co-precipitation [7]. Recently, solid-phase extraction has achieved an increasing application because of its simple procedure, higher preconcentration factor, and rapid phase separation [8], as well as its simplicity, rapidity, short analysis time, low consumption of solvents and high enrichment factor [5].

Numerous substances have been proposed and applied as adsorbent for determining low concentrations of indium, including chelating resins [9], activated carbon [10], synthetic polymers [11], naphthalene [4, 12], silica gel [13] and polyurethane foam [14]. The chelating resin column method has been recently used as a preconcentration and separation technique, because this technique requires only a small amount of sample (<100 mL) and can minimize contamination from various vessels and airborne particles. Many substances have been proposed and applied as chelating resin for determining low concentrations of indium, such as iminodiacetic acid resin [15], chitosan [16], diphosphonic acid resin and aminophosphonic acid resin [17]. Due to the complexity of urine matrix and low concentration of In(III) in human urine, chelating resin capable of determining low concentrations of indium in biological matrices is few reported. For example, Fortes *et al.* investigated the indium absorption onto four ion exchange resins, iminodiacetate, diphosphate, sulfonate, and aminophosphate [17]. Their experimental result indicates that the chelating resin containing iminodiacetic acid groups with best performance for purification of the indium sulfate solution, although the absorption is only 40% for indium [11].

This study is to provide the efficiency separation and preconcentration of trace indium from urine using Chelex-100 resins as an ion exchange resin. Several parameters governing the absorption recovery including pH of sample solution, volume of sample solution are investigated. In addition, the proposed method is applied to the analysis of real urine sample, and its accuracy is validated by the analysis of the standard reference materials.

II. EXPERIMENTAL

A. Instrumentation

The indium (III) content in human urine was determined by GFAAS using a Perkin-Elmer Analyst 300 atomic absorption spectrometer (Perkin-Elmer, USA) fitted with a deuterium arc background corrector, an AS-70 autosampler and an HGA-800 graphite furnace. The detection wavelength (nm) for indium was 303.94 nm with a spectral bandpass of 0.7 nm. Pyrolytic graphite-coated graphite tubes (Perkin-Elmer, Part No. B3 001264) with a platform were

used throughout the experiments. High-purity argon gas (99.996%) was the purge and sheath gas. A Mettler AG 245 microbalance (Mettler-Toledo, Inc., Switzerland) with 10 µg readability was used to weigh the reagents.

B. Reagents

The reagents used in this study included suprapure grade HNO₃, potassium phosphate, dipotassium hydrogen phosphate, and ammonium acetate, were of analytical reagent grade purchased from Merck (Darmstadt, Germany). Chelex-100 and Amberlite IRC-50 were obtained from Sima (Sigma-St. Louis, USA). Duolite GT-73 and Celite 545-AW were obtained from Supelco (Bellefonte, PA, USA). The standard In(III) stock solution with a concentration of 1000 µg/L was atomic absorption grade (Merck). Other standard stock solutions with a concentration of 1000 mg/L, and used in interference studies, including As(III), Cd(II), Cu(II), Cr(II), Pb(II), Mo(III), Al(III), Zn(II), Mg(II), Mn(II), Fe(III), Ca(II), Ni(II) and Tl(III), of atomic absorption grade (Merck, Darmstadt, Germany). Sn(II), of atomic absorption grade, was purchased from J.T. Baker. Calibration standard solutions of In(III) were prepared simply by diluting In(III) standard stock solutions with 0.2% HNO₃.

All glassware and polyethylene bottles were soaked in 10% HNO₃ for cleaning, rinsed three times with de-ionized water, and stored in a sealed container to avoid contamination. De-ionized water produced by using a Millipore Milli-Q apparatus (Milford, MA, USA) was used for all solutions and dilutions. Sodium phosphate buffer (0.1 M) was prepared by mixing an appropriate amount of potassium phosphate and dipotassium hydrogen phosphate in D.I water, to result in a solution of pH 6.5.

C. Urine collection

Urine specimens were obtained from non-occupationally exposed volunteers, aged 20-30 years, who were students from the Department of safety, health and environmental engineering at Hungkuang University. Six students supplied urine in 250 mL polypropylene bottles and delivered the urine to the laboratory on the sampling morning. Urine specimens were filtered through a 0.45 µm Nylon-66 filter, and concentrated nitric acid (1 mL of acid dL⁻¹ of urine) was added to the aliquots that were stored at 4°C for analysis within a maximum of two weeks. Urine samples that were not analyzed during this period were frozen at -20°C. Each urine specimen was corrected by enzymatic creatinine determination.

D. Column Extraction

Chelex-100 resin was soaked in 0.1 M potassium phosphate buffer overnight before being packed into a column in order to buffer the resin at pH 6.5. The size of the column used in this study was a mini-column (15 cm x 0.46 cm I.D.), commercially available (Supelco, Bellefonte, PA, USA). The columns were prewashed with 2 M HNO₃ followed by de-ionized water, and then partially filled with 1.2 g (wet weight) of Chelex-100 resin. Then, the column was conditioned with 5 mL of 0.1 M potassium phosphate buffer (pH 6.5).

In this study, the optimum conditions for column extraction by investigate the flow rates of the loading solution and sample volumes. 1.5 mL of 0.1 M HNO₃ solution for elution after the indium ions in urine were adsorbed onto the column at a flow rate of 1.0 L/min.

E. Quantification by GFAAS

Upon elution by HNO₃, the levels of indium in urine were determined by GFAAS, and those in the standard and blank were determined using the same procedures as the samples. Quantitative results were obtained from the optimized pyrolysis and the atomization temperature program developed in this study, as indicated in Table 1. For each measurement, aliquots of 10 µL samples were autoinjected onto the pyrolytically coated platform located in a pyrolytic graphite tube. The sample solutions and corresponding blank solutions were analyzed to determine their integrated absorbance (A_i). All measurements were made in triplicate based on A_i. The collection efficiency is the percentage recovery in the elute, and calculated using the following equation (1)

$$\text{Recovery (\%)} = \frac{\text{mass of collected metal}}{\text{mass of loaded metal}} \times 100\% \quad (1)$$

F. Calibration and quality control

To validate the proposed method, a freeze-dried human urine standard reference material, Seronorm™ Trace Elements Urine (Ref 201205 Lot 0511545) was prepared commercially (Nycomed, Oslo, Norway) and used as a quality-control standard.

III. RESULTS AND DISCUSSION

A. Selection of ion exchange resin

To obtain quantitative recovery of indium ion on ion exchange resin, four common resins were tested including Chelex-100, Amberlite IRC-50, Duolite GT-73 and Celite 545-AW. The effect of different ion exchange resins on the adsorption recovery for the In(III) in urine was studied and shown in Fig. 1. It was found that the Chelex-100 was the best ion-exchange resin in this study.

B. Effect of pH

In order to optimize the pH for maximum removal efficiency, the experiments were conducted with 100 µg/L of indium solution containing 1.2 g of Chelex-100 in the pH range 2.0-8.0. The effect of the pH on the removal of In(III) by chelating resin was shown in Fig. 2. When the surface of Chelex-100 carries negative charges, it begins to adsorb In(III) ions. The recoveries of indium at the pH range of 6-7 were found quantitative (>95%). Therefore, the optimum pH was set as 6.5 for the following experiments.

C. Desorption and enrichment factor

In this study, the adsorption recovery was approximately 100% at a loading flow rate of 1.0 mL/min. This result is consistent with Kim et al., study [18]. Therefore, the optimum flow rate was selected as 1.0 mL/min.

In order to decrease other interference for indium determination by GFAAS, HNO₃ was chosen as elute for this study. Meanwhile, the optimum concentration of elute,

various concentration HNO_3 were studied for the elution of retained In(III) from Chelex-100 resin. Quantitative recoveries of the indium absorbed on the resin were found using 0.1 M HNO_3 at a flow rate of 1.0 mL/min, as shown in Fig. 3. The effect of elute volume on the recovery of indium was also studied by keeping the HNO_3 concentration of 1.0 mol/L, it was found that the optimum recoveries could be obtained with 1.5 mL HNO_3 . Therefore, the volume of 1.5 mL eluent was used for following experiments.

The influence of the sample volume on recoveries of indium was also examined. For this purpose, indium was preconcentrated from volumes of 10, 20, 25, 50 and 100 mL of sample solution containing 200 $\mu\text{g/L}$ In(III) by applying the procedure mentioned above. The maximum sample volume can be up to 50 mL with 95% recovery and enrichment factor was 33 by 1.5 mL HNO_3 elution [6].

D. Interference effects

Due to the high selectivity achieved in GFAAS, the only interferences in this study were related to the preconcentration step. Several interfering ions that would be absorbed to Chelex-100 resin and be eluted were examined using the optimized preconcentration procedure. Metal ions were added individually to a solution as their nitrate or nitrate or chloride salts. The tolerance limit of interfering ion was set as the amount of ion required to cause a 5% error in the determination of indium. As shown in Table 2, the major cations and anions present in human urine samples had no obvious influence on the determination of In(III) by the optimized pre-concentration procedure.

E. Method validation and analytical performance

In this study, the proposed procedure was applied to determine indium concentrations in six urine specimens from healthy volunteers. Experimental results demonstrated that the levels of indium in urine were lower than the detection limit (DL) of this proposed method. After the urine samples were spiked with 10 ng ml^{-1} and 20 ng ml^{-1} indium, the satisfactory recoveries were obtained. Indium concentration determined as mean of six determinations at 95% confidence level was shown in Table 3.

From measurements made under the optimum conditions described above, the calibration graph was linear in the range 20-250 $\mu\text{g/L}$. The calibration equation was with a correlation coefficient of 0.9992. The detection limit (evaluated as concentration corresponding to three times the standard deviation of 11 runs of blank solution) of this method for In(III) was 2.75 $\mu\text{g/L}$, and the relative standard deviation (RSDs) 4.9% ($n=6$, $C=20 \mu\text{g/L}$).

Compared to other method for the trace indium determination in urine by mean of GFAAS [19], this developed procedure was more simple and reliable. Miyaki *et al.* monitored of indium in urine samples using microwave digestion as sample treatment method prior GFAAS determination [19]. However, the use of hazardous acids was generally required for microwave digestion. The detect limit of the In(III) in urine was 0.4 $\mu\text{g/L}$, which was comparable to our result.

IV. CONCLUSION

Based on the obtained results, Chelex-100 resin is an effective resin for preconcentration and separation of trace In(III) from urine samples. Furthermore, the analytes are eluted from minicolumn that contained only dilute HNO_3 solution, producing sufficiently clean solution for GFAAS determination, without the need for further cleanup. This method is suitable for preconcentration of In(III) in urine with low detection limit, high selective absorbability and high precision. Additionally, the effects of coexisting interfering ions in the quantitative analysis of In(III) could be eliminated by the selective absorption and elution. Hence, this method can be applied to quantity of trace In(III) in urine or other biological samples.

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Table captions

- Table 1 Optimized furnace program for analysis of indium in aqueous solution
- Table 2 Tolerance limits for coexisting ions in adsorption of In(III) from urine
- Table 3 Analytical result of In (III) in real samples and standard reference material (n = 6).

Figures captions

- Fig.1. Effect of various ion exchange resins on the percent recovery of In(III) from urine
- Fig.2. Effect of pH on adsorption of In(III) from Chelex-100 resin.
- Fig.3. Effect of elute concentrations on the desorption recovery of retained In(III) from Chelex-100 resin.

TABLE 1 OPTIMIZED FURNACE PROGRAM FOR ANALYSIS OF INDIUM IN AQUEOUS SOLUTION

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Ar flow rate (ml min ⁻¹)
Drying	110	5	15	300
Pyrolysis	800	5	30	300
Pre-cool down	20	1	15	300
Atomization	1900	0	5	0
Cleaning	2400	1	5	300

TABLE 2 TOLERANCE LIMITS FOR COEXISTING IONS IN ADSORPTION OF

IN (III) FROM URINE	
Coexisting ions	Foreign ion to In(III) ratio
Al ³⁺ , Cu ²⁺	125
Cr ³⁺ , Cd ²⁺	125
Fe ³⁺ , Mo ³⁺	125
As ³⁺ , Pb ²⁺ , Mg ²⁺ , Zn ²⁺	1000
Ni ²⁺	250
Na ⁺ , K ⁺ , Cl ⁻ (added as KCl, NaCl)	1000
Mn ²⁺	125
Tl ³⁺ , Sb ³⁺	500
PO ³⁻ (added as Na ₃ PO ₄)	250

TABLE 3 ANALYTICAL RESULT OF IN (III) IN REAL SAMPLES AND STANDARD REFERENCE MATERIAL (N = 6).

Added (µg L ⁻¹)	Found (µg L ⁻¹)	Recovery (%)
real samples		
0	ND	--
10	9.49± 0.08	94.9
20	20.6± 0.05	103.3
SRM		
0	ND	--
10	9.26 ± 0.04	92.6
20	19.2 ± 0.06	95.9

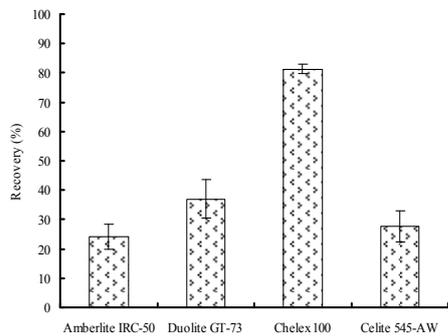


Fig.1. Effect of various ion exchange resins on the percent recovery of In(III) from urin

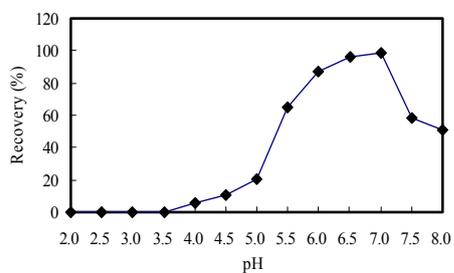


Fig.2. Effect of pH on adsorption of In(III) from Chelex-100 resin.

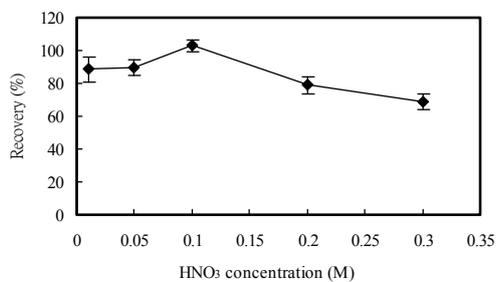


Fig.3. Effect of elute concentrations on the desorption recovery of retained In(III) from Chelex-100 resin.