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RESEARCH ARTICLE

Extraction of trace elements in human fluids using cation exchange resin (chelex-100) columns for ICP-MS analysis

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Abstract: A successful metal ion extraction method was developed using cation exchange resin packed disposable micropipette tip columns for the extraction of trace metal ions in human fluids. Using this method, concentrations of Mn, Ni, Cu, Zn, Cd and Pb ions in human urine were determined by inductively coupled plasma mass spectroscopy (ICP-MS). The main objective of this study was to develop a technique to overcome spectral interferences in ICP analysis of trace elements in biological fluids mainly due to salts present in the samples. The amount of resins packed to the column, amount of eluent needed to extract trace elements, and particle size of the resin were examined to determine conditions for metal extraction. The validity of this technique was evaluated by calculating recoveries of trace element concentrations of above elements in certified standard urine samples and by using the technique for trace metal spiked urine samples. The results revealed that Pb, Cd, Zn, Cu and Mn had good recoveries (about 95%) while Ni & Co had about 80% recovery. It was also found that the recoveries of trace elements depended on the concentrations of relevant elements in the samples.

Keywords: Trace element, chelex-100 resin, ICP-MS analysis

Introduction

Determination of trace elements in human fluids such as urine and blood has attracted considerable attention and interest in medical and biological sciences (Lyengar, 1989; Versick and Cornelis, 1999; Haraguchi, 1999; and Kazumi and Haraguchi, 2000). In the recent past, many techniques such as X-ray fluorescence, neutron activation, isotope dilution mass spectrometry, UV-VIS spectrophotometry, atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) have been developed for trace element analysis (Kazumi et al. 2000). Among them, ICP-MS had been extensively applied to analyze biological material and fluids, because of its capability for rapid multi-element detection over a wide range of concentrations with very low detection limits and easy operation (Harald and Pulvermacher, 2005; Obata et al., 1993; Michael et al., 1989; Michael et al., 1989). However, major constituents that exist in digested biological samples such as inorganic salts, cause difficulties in the analysis of trace elements (Yang et al., 1990; Sun and Huang, 2003; Kasumi et al., 2000; Jorgelina et al., 2002). These inorganic salts result in signal suppression or enhancement, clogging of the sampling interface orifices and spectral interference of poly atomic and doubly charged ions (Wassana et al., 2007).

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In order to overcome interference due to the presence of high chloride ion concentration in the sample and to pre-concentrate the metal ions, different types of chelating agents such as APDC (Ammonium Dithio Carbamate), FREON (Trichloro,Trifluoro-Ethane, $C_2Cl_3F_3$) (Jorgelina et al., 2002 and Harald et al., 2005), and EDTA (Ethylenediamine tetraacetic acid) have been used to extract trace metals from human blood. The use of ion exchange resin is another option in order to overcome the problem of interference due to the salt matrix. It is well known that Chelex-100 cation-exchange resin has been employed in extracting cations from salt media in environmental samples such as sea water (Pai et al., 1990.) and biological samples such as human fluid (Sun and Huang, 2003). Chelex-100 resin has been applied in its different cationic forms such as sodium and ammonium form (Pai et al., 1990). However, several problems still exist in the application of chelation resin to separate trace metals from real samples with a complex matrix. Among them, the major problem is the recovery of trace elements that is severely affected by several factors such as pH of the medium, flow-rate in column systems, amount of the resin used and the particle size of the resin. Especially, the efficiencies of trace metal extraction is critically pH-dependent and the optimal pH may vary from method to method and from metal to metal (Figura and McDuffie, 1980). In multi elemental analysis of biological fluids, it is important to optimize environmental conditions for each element. Certain conditions that give very high recovery for one element may not be optimum conditions for another element. However, trace metal separation from environmental or biological samples before instrumental analysis is not practically used yet.

The purpose of this study was to develop and to investigate the applicability of metal ion extraction techniques using resin columns for the determination of the concentration of trace elements in human fluid by ICP-MS. The trace elements selected for the analysis were Mn, Co, Ni, Cu, Zn, Cd, and Pb in human urine obtained from patients in the Taipei veterans hospital, Taiwan (Republic of China). The effect of pH of the metal solution, amount of resin loaded into columns, the particle size of resin and eluent volume needed to elute absorbed metal ions were optimized.

Materials and Methods

Chemicals & Reagents

All reagents of analytical or higher grade and water used for all preparations were first treated with reverse-osmosis and further treated with mixed-bed ion exchanger unit (MiliPOR 10 PLUS). Nitric acid (Merk, 60%, Trace Pure) was diluted to required amounts to prepare 2N diluted solution and ammonium hydroxide (Merk Ultra-Pure grade) was used to prepare 1N diluted solution. These solutions were stored in pre-cleaned Teflon bottles. Standard urine reference was purchased from Sernorm Trace Elements (Norway). All plastic containers and pipette tips used for the experiment were washed with detergent, distilled water and soaked in a 10% diluted nitric acid bath for over one week and rinsed with de-ionized distilled water before use. All experiments were carried out in a class-100 clean room.

Preparation and purification of resin columns

Chelex-100 chelating resin (100-200 mesh and 200-400 mesh-analytical grade) were purchased from Bio-rad Laboratories (USA). Resin of 100-200 mesh was used throughout the experiment except for comparison for which resin with particle size 200-400 mesh was used. Ion-exchange columns were prepared by 10 ml-polyethylene auto-pipette tips of 10 cm in length and 1 cm diameter in the wider side and 0.5 mm diameter hole in the narrow side. A pre-cleaned glass wool bud was placed near the tip of the column to avoid resin passing outside. Weighed amounts of the resin, originally in its sodium form at the delivery, was loaded into the pre-cleaned column and washed with 10 ml of 2N nitric acid followed by excess of distilled de-ionized water (DI water). Then the resin containing column was converted into the ammonium form by washing with 5 ml of 1 N ammonium hydroxide solution. The excess ammonium hydroxide was washed out with water until the pH of drained water was around 7-8.

The previously pH-adjusted sample was introduced into the column drop-wise without disturbing the resin bed and was allowed to drain out by gravity. Sorbed metal ions were extracted by draining out with 10 ml of 2N nitric acid. The eluent was collected into a 10 ml volumetric flask and the volume was adjusted with 2N nitric acid. Analysis of trace elements was conducted by using ICP-MS (ELAN 5000, Perkin Elmer) spectrometer immediately after the sample preparation was completed.

Preparation of buffer solutions

Maleic acid/ammonia buffer solution was prepared following the procedure, as briefly explained here. A sample weighing 58.01 g of maleic acid (99%, Ridel HPLC grade) was dissolved in 600 ml of water and pH was adjusted to required values of 9.0, 8.0, 8.5, 7.5, 7.0, 6.5, 5.0, 4.5, etc. using concentrated ammonium hydroxide and the volume of the solution was set to 1.00 liter. The solution was purified by passing through chelex-100 resin packed column and then, stored in a Teflon bottle. Measurements of pH were carried out by using a pH meter (Orion 720A) equipped with a combination pH electrode that was calibrated with standard pH buffer solutions of pH 4.00 and pH 9.00.

Sample digestion and preparation and storage

Urine samples obtained from patients were collected into pre-cleaned PE vials and stored at -20^{o}C immediately after collection until they were taken out for sample preparation. 4 ml of urine sample at room temperature was treated with 2 ml of concentrated nitric acid. Then the samples were digested in a microwave oven (CEM, MDS 2000) under 180^{o}C for 20 minutes. After digestion, colourless solutions were obtained. After cooling down, the pH of digested samples were adjusted to required values (6.5, 7.5, 8.0, 9.0) by adding necessary amounts of concentrated ammonium hydroxide solution and 2N ammonium hydroxide with 2.0 ml of ammonia/malate buffer solution. Urine samples from non-patients, patients and of the standard reference were treated by the same procedure.

Results and discussion

Extraction of trace elements using Chelex-100 packed home-made columns

Pre-concentration of trace elements from environmental samples such as seawater, using chelex-100 resin has been reported in previous studies (Baffi et al., 1992) but the possibility of application of this technique for biological samples has not received much attention. Certain studies reported trace elements extraction by resins in batch systems (Baffi et al., 1992 and Bhindi and Chiswell B., 1992). However, with small volumes of samples such as body fluids, the element extraction using resin in batch systems is not practical and instead, the column technique could be used. Moreover, the column technique is easier than continue-mix techniques and therefore, in this work, column technique was applied. Plastic auto-pipette-tips were used to prepare resin packed columns because they are inexpensive and considered as disposable materials. In all extraction experiments resin columns were used only once.

Effect of the volume of HNO_3 acid as an eluent

The volume of HNO_3 for eluting the sorbed metal ions was identified as an important factor. The techniques for eluting the sorbed metal-ions from resins were thoroughly studied to find out the optimum conditions. Table 1 demonstrates that when the volume of eluent was increased, the recovery also increased. However, under the experimental conditions used, it was recognized that 10 ml of 2N HNO_3 was suitable for metal ion elution. Previous work (Kazumi et al., 200) has reported that even 30 ml of 2.5N HNO_3 could elute only 70% Cu and Cd in sea water, under a flow rate 2.5 ml min^{-1} through resin column. In this work the flow rate was very low (6-1ml min^{-1}) and retention time in the column is long enough to release the sorbed trace metal ions. However use of a large volume is not suitable because the concentration of the final extracted solution becomes lower due to dilution.

Table1. The recovery of trace element in 25 ppb standard solution in pH 6.5 medium, extracted to 1.00 g of chelex-100 resin (200-400 mesh) eluted by different volumes of 2N nitric acid.

Trace element	Recovery (%) , when eluted by different volumes of 2N HNO_3		
	2.00 ml HNO_3	5.00 ml HNO_3	10.00 ml HNO_3
Mn	15.64	50.62	105.80
Co	10.23	45.10	82.32
Ni	7.56	60.75	101.59
Cu	20.05	35.32	86.44
Zn	18.50	70.55	111.33
Cd	3.25	27.37	85.61
Pb	18.65	63.44	93.29

In both types of resin systems, the recovery could be enhanced with increasing resin amount. However, as shown in Table 2, there was a decrease in recovery of certain elements, when the amount of resin was increased from 1.00 g to 2.00 g. In the case of different particle size or different weight of resin, the flow rate is another important factor because when the resin with different particle sizes are packed into micropippet tips the spaces between particles vary. Average inter particle space in a column packed with large particle is greater than that in a column packed with small particles and therefore, the liquid flow rate is higher in a column with large particles. In addition, column preparation condition and swelling of chelex-100 resin cause variations in the flow-rate. The swelling of chelex-100 resin depends on the pH. In an alkaline medium, the observed volume increase was nearly twice its original volume. In the present work, flow-rate in resin-packed column varied from 0.6 ml min^{-1} to 0.1 ml min^{-1}. However, according to Table 2, variation of the recovery was not so high when the resin with small particle size (200-400 mesh) was utilized because of its comparatively larger surface area.

Table 2. The variance of recovery due to different amounts of Chelex-100 resin with mesh size 100-200 mesh and 200 -400 mesh. The trace element concentration in the solution - 25 ppb and pH of the solution was 6.5

Element	Recovery of trace metals (%)							
	0.5g of resin		1.0 g of resin		1.5 g of resin		2.0 g of resin	
	100-200 mesh	200- 400 mesh	100-200 mesh	200- 400 mesh	100-200 mesh	200- 400 mesh	100-200 mesh	200- 400 mesh
Mn	56.26	92.05	79.88	105.80	82.02	94.55	85.39	87.88
Co	39.25	75.27	45.65	82.32	60.86	84.74	68.91	86.82
Ni	29.25	91.35	49.50	101.59	55.56	109.07	58.43	97.66
Cu	50.34	88.89	75.32	86.44	80.24	118.26	82.45	116.28
Zn	60.05	103.02	65.04	111.33	75.94	127.95	85.98	131.35
Cd	56.54	83.47	58.30	85.61	69.32	89.36	72.56	83.76
Pb	68.75	94.34	76.98	93.29	85.50	94.84	92.74	102.17

Several investigators have reported (Figura P. and McDuffie B., 1980,) that the effect of pH is an important factor for the binding of polyvalent metal ions into the amino-diacetate groups which act as chelating groups in the chelex-100 resin. However, the efficiency of the process depends on each possible combination of the loaded volume of sample, eluent amount, analyte (Pai et al., 1990) species, resin type and effect of pH adjusted by ammonium hydroxide/ maleic acid, as explained by Pai et al. (1990). Maleic/ammonia buffer system is suitable for pH adjustment in the range of 2.0 - 9.0. The other advantages are that the maleic acid /ammonium buffer system does not form strong complexes with trace elements and has high chemical stability and good solubility.

Effect of particle size and the amount of the resin

Chelex-100 of 100-200 and 200-400 mesh types were used for the comparison of the efficiency of extraction of trace elements. Weighed amounts of 0.5, 1.0, 1.5 and 2.0 g of resin were used from both types of resin (100-200 and 200-400 mesh). The pH of the solution of 25 ppb trace elements was adjusted to 6.5. There was a higher recovery of trace elements in the elusion from the small particle size resin (mesh 200-400) in practice. Because of having a larger specific surface area, resin with small particle size can extract larger amounts of trace metal ions from the sample. Therefore, as shown in Table 3 higher recoveries of all the trace elements could be obtained by using the resin with small particle size (200-400 mesh). Table 3 shows the recovery of trace elements in 50 ppb solution under different pH values. Resin amount used was 1.00 g and sorbed metals were eluted using 10.00 ml of 2N HNO_3 acid. The relative standard deviations (RSDs) for recoveries are given as well. It is clear that all trace metal ions had low recovery at lower pH values. As shown in Table 3, the maximum recovery of both Co and Ni is about 82%. When the pH of the medium is lower than 6, all detected trace metals had low recovery. Therefore, pH 7.5 was selected as the working pH for trace metal separation from the samples. However, the recovery of each trace metal varied when the initial concentration of the metal solution was changed to 10 ppb (data not shown). The recovery of Ni was found to increase to approximately 100% in a pH 8 medium. The relative standard (RSD) values for the recoveries at low concentrations (10 ppb) were higher than those values at higher concentrations (50 ppb). It may be due to higher uncertainty for recoveries of trace metal ions at low concentrations. Considering the results shown in Table 3, reasonable recoveries for many trace elements could be found at pH 7.5 and therefore, pH value was selected as an optimum condition for metal separation from real samples by chelex-100 packed columns. However, experimentalists have used different pH values for the extraction of trace metals from different types of samples to chelating resin medium.

Table 3. Recoveries of trace metal solution (50 ppb) extracted by chelex-100 resin column (1g of resin) and eluted by 10 ml of 2M HNO_3 and relative standard deviations (RSDs), calculated using recovery for four replicates.

Trace metal	**Recoveries (%) and RSDs under different pH**						
	pH 9.0	**pH 8.0**	**pH 7.5**	**pH 6.5**	**pH 6.0**	**pH 5.0**	**pH 4.5**
Mn	98.25 ± 3.46	96.65±2.06	95.30±1.22	95.01±1.05	85.10±4.4	80.02±2.91	50.52±2.9
Co	89.65±1.85	87.00±3.76	85.21±8.92	80.21±1.17	51.32±6.8	45.24±3.29	20.65±4.37
Ni	86.45±3.47	85.53±11.92	84,24±5.98	80.32±9.24	70.56±5.48	43.03±1.39	31.54±5.82
Cu	108.25±3.80	105.55±7.95	101.12±4.21	95.40±6.01	65.65±5.21	45.34±5.76	30.21±7.45
Zn	118.35±1.94	112.32±5.70	105.21±5.54	98.51±3.53	71.24±0.7	42.34±4.30	35.65±3.29
Cd	110.43 ±4.17	107.56±9.52	101.56±8.05	95.32±6.55	71.45±8.79	41.28±3.25	33.26±4.26
Pb	115.32±8.36	109.40±1.78	104.43±3.75	102.32±2.1	75.54±2.72	40.43±7.7	20.25±3.59

Table 4. Recovery of trace elements from urine of healthy persons. Metal ions were extracted from urine to ion exchange columns and analyzed by ICP-MS.

Trace element	Co	Ni	Cu	Zn	Cd	Pb	Mn
Spiked concentration (ppb)	50.00	75.00	75.00	50.00	50.00	50.00	75.00
Recovery (%) ± RSD	88.88 ±7.50	82.28 ±6.40	96.89 ±5.6	107.05 ±6.40	87.05 ±7.50	91.45 ± 4.70	91.35 ± 8.53

Table 5. Concentrations of trace metals of certified standard urine samples separated by chelex-100 anion exchange resin column and analyzed by ICP-MS.

Trace Element	Co	Ni	Cu	Zn	Cd	Pb	Mn
Certified range (µg L^{-1})	10.00 - 11.00	41.50 - 39.30	25.60 - 29.20	980.00 - 1000.00	4.90 - 5.50	90.00 - 97.00	55.50 - 65.80
Detected (µg L^{-1})	8.41	40.23	27.30	1020.68	4.22	94.02	58.65

Analysis of trace elements in metal ions spiked samples using chelex-100 pact columns

Urine samples obtained from healthy persons were spiked with known amounts of a trace metal standard and were digested by following a previously described procedure. Recoveries of spiked trace elements were calculated by the standard addition method. Tables 4 and 5 illustrate the recovered concentrations of metal ions spiked urine samples and standard reference urine samples. Recovery of trace metals from spiked urine samples is slightly lower than that from trace metals in distilled de-ionized water, which may be due to small amounts of organic matter remaining even after the acid digestion which trap some trace metals (Yang 1985). The results of the certified standard reference urine sample analysis (in Table 5) after separation of trace metal ions by chelex-100 columns, indicate that the measured concentrations of Ni, Cu, Zn, Pb are within the range of certified values and Co ions had slightly lower recovery values as Co ions may have low chelating ability with resin under the experimental conditions. However, Hg, Cr, and As in certified standard urine samples could not be detected by this technique (data not shown) because chelex-100 resin can be applied only for separation of divalent cations.

Conclusion

In this study, we have shown that columns packed with chelex-100 cation resins could be used to overcome the matrix effect in real sample analysis, by chelating of metal ions to resins followed by eluting them to acid solution. The optimum amount of resin, eluent volume, size of resin granules and pH were determined. The recoveries of trace metals have been found to depend on factors such as, amount of resin used, the volume of eluent (2N HNO_3 acid) and size of resin granules. According to results obtained, the developed method is successful for use of chelex-100 resin to quantitative analysis of trace metals Co, Cu, Cd, Ni, Pd and Mn in human urine samples.

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