



Selective Solid-Phase Extraction of Zn(II) by An Ion-Imprinted Polymer from Environmental and Biological Samples

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(Received: 27 August 2011;

Accepted: 21 March 2012)

AJC-11207

A new ion-imprinted polymer material was synthesized by copolymerization of acrylamine as monomer, pentacrythritol triacrylate as crosslinking agent and 2,2'-azobisisobutyronitrile as initiator in the presence of zinc(II)-2-ethylhexyl benzimidazolyl sulfide complex. The ion-imprinted polymer was used as sorbent in a solid-phase extraction column. The effects of sampling volume, elution conditions, sample pH and sample flow rate on the extraction of zinc ions from environmental and biological samples were studied. The maximum adsorption capacity and the relative selectivity coefficients of imprinted polymer for Zn(II)/Co(II), Zn(II)/Cu(II) and Zn(II)/Ni(II) were calculated. Compared with non-imprinted polymer particles, the ion-imprinted polymer had higher selectivity for Zn(II). The detection limit for 100 mL of sample was $0.5 \mu\text{g L}^{-1}$ using flame atomic absorption spectrometry. The analytical results for the certified reference samples (GBW07603) were in a good agreement with the certified value. The relative standard deviation for eleven replicate sample of $8 \mu\text{g L}^{-1}$ level is 3.1 %. The developed method was successfully applied to the determination of trace zinc in environmental and biological samples with satisfactory results.

Key Words: Ion-imprinted polymers, Solid-phase extraction, Zinc ion, Flame atomic absorption spectrometry.

INTRODUCTION

Zinc is one of the most important essential trace elements in human nutrition. Deficiency of zinc in the human body leads to several disorders, but an excessive zinc intake also causes various acute and chronic adverse effects. Zinc has been reported to cause electrolyte imbalance, nausea, anaemia and lethargy^{1,2}. Because of the importance of zinc, simple and sensitive analytical methods for the determination of trace levels of zinc are required. Several analytical techniques including inductively coupled plasma-atomic emission spectrometry, inductively coupled plasma-mass spectrometry and graphite furnace atomic absorption spectrometry are available for the determination of trace metals with sufficient sensitivity for most of the environmental applications. But the required instruments are expensive, day-to-day maintenance cost is high and various types of inherent interferences appear. The analysis of Zn^{2+} by readily available and low cost flame atomic absorption spectrometry demands the use of preconcentration procedures in view of its insufficient sensitivity and matrix interference³⁻⁷. Solid-phase extraction is one of the most effective preconcentration methods because of simplicity, rapidity, high preconcentration factor and high recovery. The procedures for separation and preconcentration of zinc have been reported extensively. Nowadays, molecular imprinted

polymer (MIP) has been largely used as the solid phase extraction sorbent. For metal ions, molecular imprinting can be interpreted as ionic imprinting exactly. The high selectivity of ion-imprinted polymers (IIPs) have been prepared and used for selective solid-phase extraction and preconcentration of metal ions⁸⁻¹⁷.

In this study, an ion-imprinted polymer used for selective extraction and preconcentration of zinc ions was synthesized using acrylamine (AM) as monomer, pentacrythritol triacrylate (PETA) as crosslinker and 2,2'-azobisisobutyronitrile as initiator in the presence of zinc(II)-2-ethylhexyl benzimidazolyl sulfide (EHBMS) complex. After removal of Zn^{2+} , zinc-imprinted polymer was used for solid-phase extraction and determination of Zn^{2+} in environmental and biological samples. The proposed method presented high selectivity and possessed simple, convenient and accurate characteristics for zinc determination.

EXPERIMENTAL

A Z-2000 polarized zeeman atomic absorption spectrophotometer (Hitachi High-Technologies Corporation, Japan) was used to measure the concentration of metal ions (Zn, Ni, Co and Cu) in aqueous solution. The instrumental parameters were those recommended by the manufacturer. Hollow cathode

lamps were used as the radiation sources. The wavelengths selected were as follows: Zn 213.9 nm, Cu 324.8 nm, Co 240.7 nm and Ni 232 nm. The pH values were measured with a PHS-3C precision pH meter (REX Instrument Factory, Shanghai, China).

A peristaltic pump mode FIA-3110 (Titan Instruments Co., Ltd. Beijing, China) was used in the solid-phase extraction process. A WX-4000 closed vessel microwave system (Preekem Scientific Instruments Co., Ltd. Shanghai, China. maximum pressure 35 atm, maximum temperature 235 °C).

2-Ethylhexyl benzimidazolyl sulfide was synthesized in our laboratory¹⁸. Acrylamine(AM), azobisisobutyronitrile (AIBN) and pentacrythritol triacrylate (PETA) were obtained from Alfa Aesar.

Ultra-pure water of resistivity 18 MΩ cm obtained from a UPHW purification device (Ulupure Co. Shanghai, China) was used to prepare all the solutions. High purity hydrochloric acid and analytical grade ZnCl₂·6H₂O were purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

All glass and plastic material were thoroughly cleaned and kept into 10 % (mm⁻¹) nitric acid for at least 48 h. The material was then rinsed three times with ultra-pure water before being used. Standard stock solutions of Zn (II), Co(II), Cu(II) and Ni(II) (1000 mg L⁻¹) were obtained from the National Institute of Standards (Beijing, China). Working standard solutions were obtained by appropriate dilution of the stock standard solution.

Ion-imprinted polymer preparation: The Zn(II) ion-imprinted beads were prepared by bulk polymerization. An amount of 2 mmol of 2-ethylhexyl benzimidazolyl sulfide, 2 mmol acrylamine(AM) and 1 mmol of ZnCl₂·6H₂O was dissolved in 12 mL of methanol-chloroform mixture (1:2,v/v) and stirred for 2 h. Then, 22 mmol pentacrythritol triacrylate and 50 mg azobisisobutyronitrile were added. The polymerization mixture was cooled to 0 °C and purged with N₂ for 15 min, sealed and heated at 70 °C with stirring for 20 h. After polymerization, the solid formed was ground into fine particles. The resulting polymer was treated with 1 M HCl solution by continuously stirring for 4 h to remove zinc ions. The resultant polymer after filtration was dried at 80 °C for 30 h and sieved to get particles lower than 150 μm. In the same way, the non-imprinted polymer was also prepared without zinc ions.

Solid-phase extraction of zinc(II): The Teflon column was 2.6 cm long and 0.9 cm in diameter. The 2 μm of sieve plate was placed to prevent loss of the polymers during sample loading. Then, 300 mg of polymer (ion-imprinted polymer and non-imprinted polymer) was poured into the solid-phase extraction column. It was treated successively with 1 M HCl solution and water. The column was preconditioned by passing a blank solution and then a solution containing Zn(II) was passed through the column at flow rate of 2 mL min⁻¹ (controlled by a peristaltic pump) after adjusting pH 6. The column was washed with 10 mL distilled water and then eluted with 5 mL of 1 M HCl at a flow rate of 1 mL min⁻¹. The desorbed Zn(II) was measured by flame atomic absorption spectrometer.

General procedure of sample treatment: In order to the microwave digestion of biological samples, 0.200 g of sample was mixed with 5 mL of concentrated nitric acid in microwave digestion system. Digestion conditions for

microwave system of the samples were applied as 5 min for 400 W. Blanks were prepared in the same way as the sample. After cooling, the preconcentration procedure given above was applied.

For water samples, river water sample was collected from Tanglang chuan River (Kumming, P.R. China). The water sample was immediately acidified by adding several drops of nitric acid and filtrated with 0.45 μm filter. Then the preconcentration procedure given above was applied to the samples.

RESULTS AND DISCUSSION

Adsorption capacity of the imprinted polymers for Zn(II):

0.3 g of imprinted or non-imprinted sorbent was equilibrated with 50 mL of various concentrations of Zn(II) solutions at pH 6 for 4 h. The concentrations of Zn(II) in solution were measured by flame atomic absorption spectrometry and the adsorption capacity of ion imprinted polymer or non-imprinted polymer for Zn(II) ions were calculated by following equation:

$$Q = \frac{(C_0 - C_e)V}{W}$$

where, Q is the loading capacity of the polymer (mg g⁻¹) C₀ and C_e are the concentrations of the ions in the initial solution and in the aqueous phase after adsorption, respectively (mg L⁻¹); V is the volume of the aqueous phase (mL); and W is the amount of polymer (g).

Fig. 1 shows the amount of Zn(II) adsorbed per unit mass of the imprinted polymer increased with the initial concentration of Zn(II). The average maximum adsorption capacity was 4.1 and 0.4 mg g⁻¹ for Zn(II)-imprinted and non-imprinted polymers, respectively. Obviously, the capacity of imprinted polymer was larger than that of non-imprinted polymer.

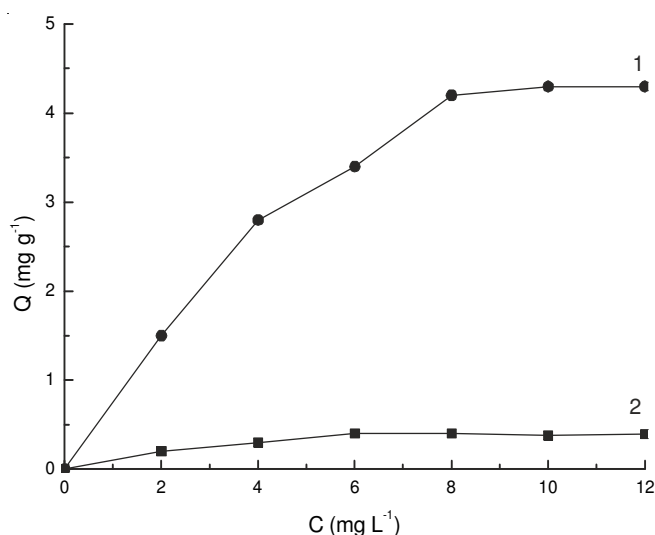


Fig. 1. Effect of initial concentration on the adsorption capacity of Zn(II)-ion-imprinted polymer (1) and Zn(II)-non-imprinted polymer (2). 300 mg of the imprinted polymer; pH 6.0; V 50 mL; temperature 25 °C

Effect of pH: The effects of the sample pH on the retention of Zn(II) ions on the ion-imprinted polymer were studied at different pH values from 1 to 7. The pH was adjusted to the desired value with aqueous ammonia and diluted HNO₃. A

volume of 50 mL of the sample containing $80 \mu\text{g L}^{-1}$ of zinc ions were passed through the ion-imprinted polymer column at a flow rate of 2 mL min^{-1} , then the solid-phase extraction column was eluted with 5 mL of 1 M HCl at a flow rate of 1 mL min^{-1} . The zinc content of the eluate was measured by flame atomic absorption spectrometry. The extraction curve of zinc as a function of the pH is shown in Fig. 2. The extraction of zinc ions were almost constant between pH 1 and 3. The percent recovery of zinc ions are sharply increased with increasing pH of the solution from 3 to about 6, so that zinc ions are quantitatively extracted at pH 6. The hydroxide in a basic solution may precipitate zinc ions. Therefore, to obtain the maximum extraction efficiency, pH of the sample solution should be carefully adjusted at pH 6.

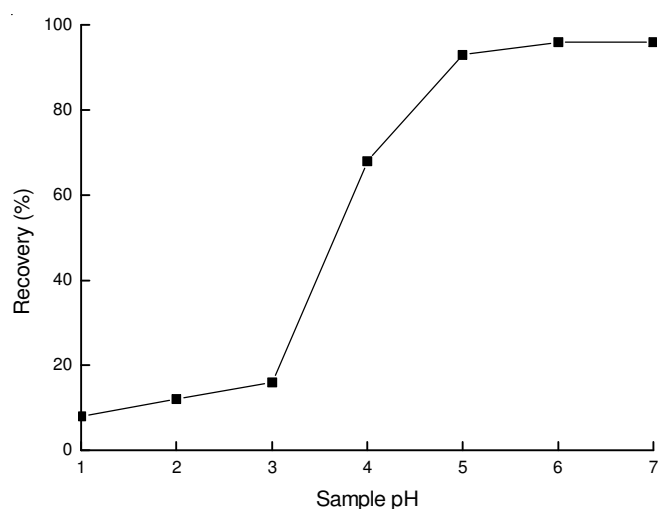


Fig. 2. Effect of sample pH on the adsorption of Zn(II) on the Zn(II)-imprinted polymer. Sample volume: 50 mL, Zn(II) concentration: $80 \mu\text{g L}^{-1}$. Sample flow rate: 2 mL min^{-1} , HCl concentration: 1 M, HCl flow rate: 1 mL min^{-1} , elution volume: 5 mL

Selectivity of the imprinted polymers: The distribution ratio, the selectivity factor of Zn(II) with respect to other ions and the relative selectivity factor were calculated as the following equations :

$$D = \frac{Q}{C_e}$$

$$\alpha_{\text{Zn/M}} = \frac{D_{\text{Zn}}}{D_{\text{M}}}$$

$$\alpha_r = \frac{\alpha_i}{\alpha_n}$$

where, Q represents the adsorption capacity (mg g^{-1}), C_e the equilibrium concentration of metal ions ($\mu\text{g mL}^{-1}$), D_{Zn} and D_{M} represent the distribution ratios of Zn(II) and other ions (mL g^{-1}). α_i and α_n represent the selectivity factor of imprinted sorbent and non-imprinted sorbent respectively.

In order to determine the selectivity of the method, competitive adsorption of Zn(II)/Ni(II), Zn(II)/Cu(II) and Zn(II)/Co(II) from their binary mixture was also investigated by batch procedure. These ions have the same charge and similar ionic radius¹⁹. In their binary mixture the two metal ions had the same concentration of $5 \mu\text{g mL}^{-1}$ and the sorbent was 50 mg.

As can be seen in the Table-1, the imprinting effect was clearly observed. The D values of the present ion-imprinted polymer for Zn(II) was large, while decreased significantly for Co(II), Cu(II) and Ni(II). The relative selectivity factor (α_r) values of Zn(II)/Ni(II), Zn(II)/Cu(II) and Zn(II)/Co(II) were 351.5, 417.1 and 463.9, respectively, which are greater than 1 (Table-1). This means that Zn(II)-imprinted sorbent had higher selectivity for Zn(II). And the results indicated that Zn(II) could be determined even in the presence of Co(II), Cu(II) and Ni(II) interferences.

TABLE-1
SELECTIVITY PARAMETERS OF IMPRINTED
AND NON-IMPRINTED POLYMERS

Metal ions	E (%) (IIP)	E (%) (NIP)	D_i (mL g^{-1})	D_n (mL g^{-1})	α_i	α_n	α_r
Zn	99.5	22.6	39800	58.4	-	-	-
Ni	25.1	14.7	67.0	34.5	594.0	1.69	351.5
Cu	14.8	9.6	34.7	21.2	1147.0	2.75	417.1
Co	18.5	13.4	45.4	30.9	876.7	1.89	463.9

The effect of different foreign ions such as CaCl_2 , Na_2SO_4 , KNO_3 and Na_3PO_4 , on the adsorption of Zn(II) was studied using the batch procedure. The results showed that 80-350-fold electrolytes did not interfere with adsorption and desorption of Zn(II), indicating that the present imprinted polymers can be suitably used as sorbents for Zn(II) in high electrolytes. The reported tolerance limit is defined as the ion concentration causing a relative error $< \pm 5\%$.

Effect of flow rate of sample solution and eluent: Effect of the sample flow rate on the adsorption of zinc ions was examined under the optimum conditions (pH, eluent, etc.). A volume of 50 mL of the sample containing $80 \mu\text{g L}^{-1}$ of zinc ions was passed through the ion-imprinted polymer column. The flow rate was adjusted in the range of $0.5\text{-}3.0 \text{ mL min}^{-1}$ using a peristaltic pump. In the test, the quantitative recoveries of the metal ions will decrease with the further increasing of the flow rate that is over 2 mL min^{-1} . Thus, a flow rate of 2 mL min^{-1} was selected in this work.

For the elution of Zn(II) from the column, three parameters, *i.e.* elution volume, eluent (HCl) flow rate and eluent concentration were investigated and optimized. Flow rate of HCl solution was the first parameter to be optimized. A volume of 50 mL of the sample containing $80 \mu\text{g L}^{-1}$ of zinc ions was passed through the ion-imprinted polymer column at 2 mL min^{-1} . Elution of the solid-phase extraction column was performed with 5 mL of 1 M HCl at different flow rates between 0.5 and 2.5 mL min^{-1} . The results showed that 5 mL of 1 M HCl at a flow rates of 1 mL min^{-1} was sufficient for 95 % recovery. In order to find optimum volume of the elution solvent, different volumes of HCl from 1 to 7 mL were investigated (Fig. 3). As can be seen from the figure, quantitative elution was attained using volumes ≥ 5 mL. The influence of HCl concentration on the elution of zinc ions from the sorbent was also examined and the results are shown in Fig. 4. Zinc ions were quantitatively eluted from the column with 1 M HCl solution.

The capacity of imprinted polymers had no considerable decrease after 40 cycles of repeated experiments. The Zn(II)-ion-imprinted polymer showed good reusability and stability towards Zn(II).

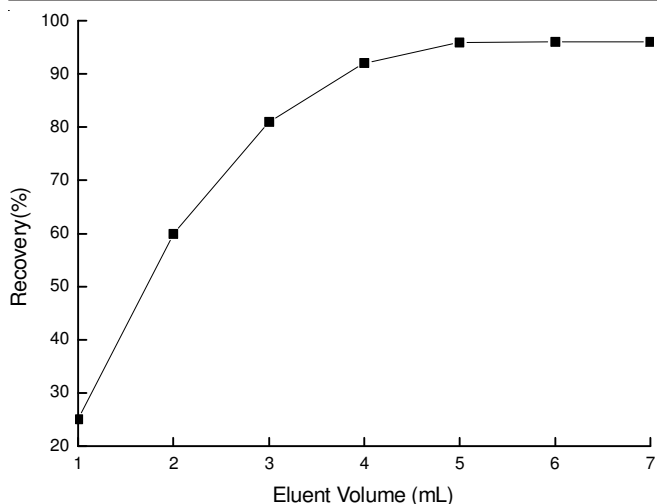


Fig. 3. Influence of eluent volume on the elution of Zn(II) from the Zn(II)-ion-imprinted polymer. Sample volume: 50 mL, sample pH 6, Zn(II) concentration: $80 \mu\text{g L}^{-1}$, HCl flow rate: 1.0 mL min^{-1} , HCl concentration: 1 M

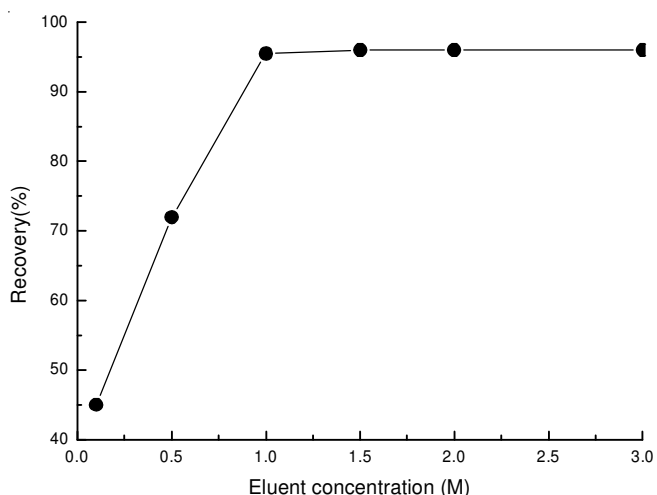


Fig. 4. Influence of HCl concentration on the elution of Zn(II) from the Zn(II)-ion-imprinted polymer. Sample volume: 50 mL, sample pH 6, Zn(II) concentration: $80 \mu\text{g L}^{-1}$, HCl flow rate : 1.0 mL min^{-1} , elution volume: 5 mL

Maximum sample volume and enrichment factor: The enrichment factor was studied following recommended column procedure by increasing volume of Zn(II) solution and keeping the total amount of loaded Zn(II) constant to $4 \mu\text{g}$. For this purpose, 20, 50, 100, 150, 200 and 250 mL of sample solutions containing $4 \mu\text{g}$ Zn(II) were passed through the column at the optimum flow rate. The results showed that the maximum sample volume could be up to 100 mL with the recovery $> 95\%$. Therefore, 100 mL of sample solution was adopted for the preconcentration of analytes from sample solutions. And a high enrichment factor of 20 was obtained.

Analytical accuracy and precision of ion-imprinted polymers-solid phase extraction: Under the selected conditions, eleven portions of $8 \mu\text{g L}^{-1}$ Zn(II) standard solutions (100 mL) were treated and analyzed simultaneously following the column procedure. The relative standard deviations of the method was 3.1 %, indicating that the method had good precision for the analysis of trace Zn(II) in solution samples.

The limit of detection given by $\text{LOD} = (3 \times \text{SD})/m$, where SD is the standard deviation of eleven procedural blanks and m is the slope of the external 1 M hydrochloric acid calibration graph²⁰, was calculated to be $0.5 \mu\text{g L}^{-1}$ for a pre-concentration factor of 20.

The method has been applied to the determination of Zn(II) in biological standard reference materials (GBW07603, Bush twigs and leaves). The results are given in Table-2.

Sample	Certified value ($\mu\text{g g}^{-1}$)	Present value ($\mu\text{g g}^{-1}$)
Bush twigs and leaves (GBW07603)	20.6 ± 2.2	20.4 ± 0.8

In order to check for possible matrix effects and investigate the applicability of the method to real sample analysis. The results are listed in Table-3. Recoveries of zinc were in the range of 94-104 %. These results indicated the suitability of the Zn(II)-ion-imprinted polymer for selective solid-phase extraction and determination of trace Zn(II) from environmental and biological samples.

Sample	Added ($\mu\text{g L}^{-1}$)	Found by present matter ($\mu\text{g L}^{-1}$)	Recovery (%)
River water	0	12.5 ± 0.2	-
	5	17.3 ± 0.2	96.0
	10	22.7 ± 0.3	102.0
Serum	0	11.5 ± 0.2	-
	5	16.2 ± 0.3	94.0
	10	21.6 ± 0.4	101.0
Human hair	0	10.1 ± 0.1	-
	5	15.3 ± 0.2	104.0
	10	19.8 ± 0.2	97.0
- not determined			

Conclusion

A new Zn-imprinted sorbent based on 2-ethylhexyl benzimidazolyl sulfide (EHBMS) ligand was developed as solid-phase extraction material for selective preconcentration of zinc ions from environmental and biological samples. The imprinted polymer has obvious imprinting effect on the imprinting ion. The values of the relative selectivity factor were found to be 463.9, 417.1 and 351.5 for Co(II), Cu(II) and Ni(II), respectively. The method shows good linearity and reproducibility. The proposed procedure was applied for the determination of zinc in environmental and biological samples.

ACKNOWLEDGEMENTS

This work was supported by Development Program of China (2011AA03A405D), Key Natural Science Foundation of China (U0937601) and Science and Technology Support Program of China (2008BAB32B10).

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