



## Selective Solid-Phase Extraction of Ni(II) by an Ion-Imprinted Polymer from Biological Samples

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A new ion-imprinted polymer material was synthesized by copolymerization of 2-vinylpyridine as monomer, ethyleneglycoldimethacrylate as crosslinking agent and azobisisobutyronitrile as initiator in the presence of Ni-5-(4-carboxylphenylazo)-8-hydroxyquinoline 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 ion extraction of Ni ions from biological samples were studied. The maximum adsorption capacity and the relative selectivity coefficients of imprinted polymer for Ni(II)/Co(II), Ni(II)/Cu(II) and Ni(II)/Zn(II) were calculated. Compared with non-imprinted polymer particles, the ion-imprinted polymer had higher selectivity for Ni(II). The detection limit for 250 mL of sample was  $0.2 \mu\text{g L}^{-1}$  using flame atomic absorption spectrometry. The analytical results for the certified reference samples (GBW10016) were in a good agreement with the certified value. The relative standard deviation for eleven replicate sample of  $5.0 \mu\text{g L}^{-1}$  level is 2.5 %. The developed method was successfully applied to the determination of trace nickel in biological samples with satisfactory results.

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

### INTRODUCTION

The analysis of nickel ion by readily available and low cost flame atomic absorption spectrometry (FAAS) demands the use of preconcentration procedures in view of its insufficient sensitivity and matrix interference<sup>1</sup>. Various preconcentration techniques including solvent extraction, coprecipitation, cloud point extraction, ion-exchange and solid phase extraction techniques have been used for the enrichment and separation of nickel ion at trace levels in various environmental samples around the world<sup>2-4</sup>.

Solid phase extraction is an attractive separation preconcentration technique for nickel ion with some important advantages (economic, rapid, higher enrichment factors). Various solid phase extraction materials have been successfully used for the preconcentration and separation of nickel ion at trace levels<sup>5-8</sup>. 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) arises from the memory effect of the polymer to the imprinted ions, *e.g.*, from the specificity of interaction of ligand with the metal ions; the coordination geometry and coordination number of metal ions. In recent years, a lot of IIPs have been prepared and used for selective solid phase extraction and preconcentration of metal ions<sup>9-15</sup>.

In this work, Ni(II)-imprinted copolymer was synthesized using 2-vinylpyridine (2VP) as monomer, ethyleneglycoldimethacrylate (EGDMA) as crosslinker and azobisisobutyronitrile as initiator in the presence of Ni-5-(4-carboxylphenylazo)-8-hydroxyquinoline (CPAHQD) complex. After removal of Ni ions, Ni-imprinted polymer was used for solid-phase extraction and determination of Ni ions in biological samples. The proposed method presented high selectivity and possessed simple, convenient and accurate characteristics for nickel determination.

### EXPERIMENTAL

A Z-2000 polarized zeeman atomic absorption spectrophotometer (Hitachi High-Technologies Corporation, Japan) was used to measure the concentration of metal ions (Ni, Co, Cu and Zn) 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: Ni 232.0 nm, Cu 324.8 nm, Co 240.7 nm and Zn 213.9 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). 5-(4-Carboxylphenylazo)-8-hydroxyquinoline (CPAHQD) was synthesized in our laboratory<sup>16</sup>. Ethyleneglycoldimethacrylate (EGDMA), azobisisobutyronitrile (AIBN) and 2-vinylpyridine (2VP) 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 nitric acid and analytical grade Ni(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O were purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

All glass and plastic material were rigorously cleaned and kept into 10 % (m m<sup>-1</sup>) 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 Ni(II), Co(II), Cu(II) and Zn(II) (1000 mg L<sup>-1</sup>) 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 (IIP) preparation:** The Ni(II) ion-imprinted beads were prepared by bulk polymerization. An amount of 4 mmol of CPAHQD, 4 mmol 2-vinylpyridine (2VP) and 2 mmol of Ni(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O were dissolved in 12.0 mL of methanol-chloroform mixture (1:1, v/v) and stirred for 2 h. Then, 32 mmol EGDMA and 100.0 mg AIBN were added. The polymerization mixture was cooled to 0 °C and purged with N<sub>2</sub> for 10 min, sealed and heated at 65 °C with stirring for 8 h. The hard bulk polymers were grounded and screened, of which the 75-150 μm size were selected as experimental sorbents. The particulates were washed with 1 M HNO<sub>3</sub> solution. Finally, the particles were washed with distilled water and dried in a vacuum oven at 80 °C for 24 h. In the same way, the non-imprinted polymer was also prepared without nickel ions.

**Solid-phase extraction of Ni(II):** The Teflon column was 2.5 cm long and 1.0 cm in diameter. The 2.0 μm of sieve plate was placed to prevent loss of the polymers during sample loading. Then, 200 mg of polymer (IIP and NIP) was poured into the solid phase extraction column. It was treated successively with 1.0 M HNO<sub>3</sub> solution and water. The column was preconditioned by passing a blank solution and then a solution containing Ni(II) was passed through the column at flow rate of 2.0 mL min<sup>-1</sup> (controlled by a peristaltic pump) after adjusting pH 7. The column was washed with 5 mL distilled water and then eluted with 5 mL of 1 M HNO<sub>3</sub> at a flow rate of 0.5 mL min<sup>-1</sup>. The desorbed Ni(II) was measured by flame atomic absorption spectrometer (FAAS).

**General procedure of sample treatment:** In order to the microwave digestion of biological samples, 0.200 g of sample was mixed with 5.0 mL of concentrated nitric acid in microwave digestion system. Digestion conditions for microwave system of the samples were applied as 4 min for 400 W. Blanks were prepared in the same way as the sample. After cooling, the preconcentration procedure given above was applied.

## RESULTS AND DISCUSSION

**Adsorption capacity of the imprinted polymers for Ni(II):** 0.2 g of imprinted or non-imprinted sorbent was

equilibrated with 50 mL of various concentrations of Ni(II) solutions at pH 7 for 4 h. The concentrations of Ni(II) in solution were measured by FAAS and the adsorption capacity of imprinted or non-imprinted polymers for Ni(II) ions were calculated by following equation:

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

where Q = loading capacity of the polymer (mg g<sup>-1</sup>) C<sub>0</sub> and C<sub>e</sub> are the concentrations of the ions in the initial solution and in the aqueous phase after adsorption, respectively (mg L<sup>-1</sup>); V = volume of the aqueous phase (mL) and W = amount of polymer (g).

As can be seen in Fig. 1, the amount of Ni(II) adsorbed per unit mass of the imprinted polymer increased with the initial concentration of Ni(II). The average maximum adsorption capacity was 2.0 and 0.3 mg g<sup>-1</sup> for Ni (II)-imprinted and non-imprinted polymers, respectively. Obviously, the capacity of imprinted polymer was larger than that of non-imprinted polymer.

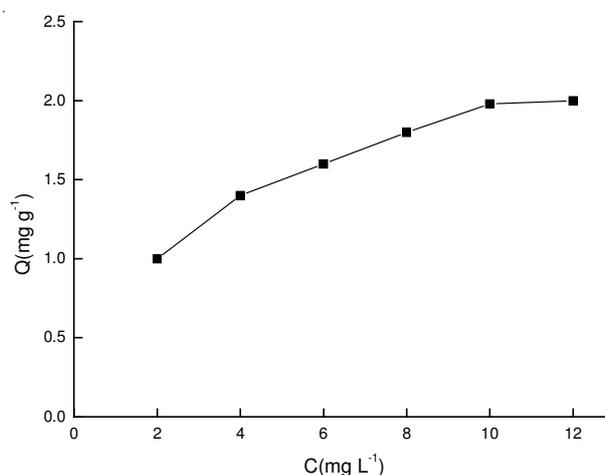


Fig. 1. Effect of initial concentration on the adsorption capacity of Ni(II)-IIP. 200 mg of the imprinted polymer; pH 7.0; V 50 mL; temperature 25 °C

**Effect of pH:** The effects of the sample pH on the retention of Ni(II) ions on the IIP were studied at different pH values from 2-8. The pH was adjusted to the desired value with aqueous ammonia and diluted HNO<sub>3</sub>. A volume of 50 mL of the sample containing 40 μg L<sup>-1</sup> of Ni ions was passed through the IIP column at a flow rate of 2 mL min<sup>-1</sup>, then the SPE column was eluted with 5 mL of 1 M HNO<sub>3</sub> at a flow rate of 0.5 mL min<sup>-1</sup>. The Ni content of the eluate was measured by FAAS. The extraction curve of Ni as a function of the pH is shown in Fig. 2. The extraction of Ni ions was almost constant between pH 2 and 5 and then increased greatly from pH 6 up to 7. In consideration of hydrolysis, pH above 8.0 was not tested. At acidic media, the donating nitrogen atoms of the ligand can be protonated, therefore, the complex is more effectively formed in a neutral solution than in an acidic solution. The per cent recovery of Ni ion is sharply increased with increasing pH of the solution from 6 to about 7, so that Ni ions are quantitatively extracted at neutral media. The hydroxide in a basic solution may precipitate nickel ions. Therefore, to obtain the maximum extraction efficiency, pH of the sample solution should be carefully adjusted at pH 7.

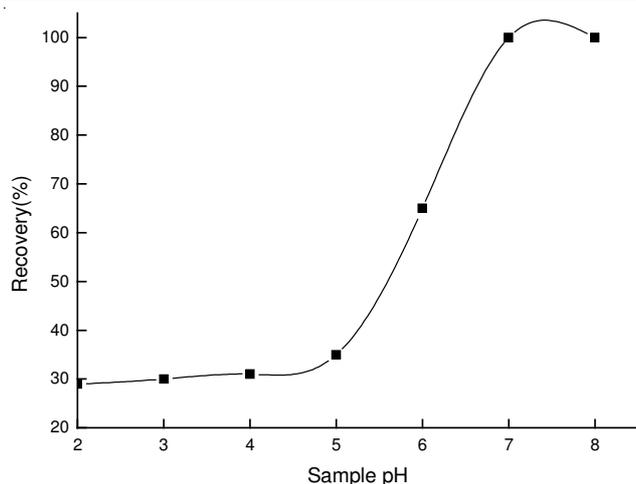


Fig. 2. Effect of sample pH on the adsorption of Ni(II) on the Ni(II)-imprinted polymer. Sample volume: 50 mL, Ni(II) concentration:  $40 \mu\text{g L}^{-1}$ . Sample flow rate:  $2 \text{ mL min}^{-1}$ ,  $\text{HNO}_3$  concentration: 1 M,  $\text{HNO}_3$  flow rate:  $0.5 \text{ mL min}^{-1}$ , elution volume: 5 mL

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

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

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

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

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

In order to determine the selectivity of the method, competitive adsorption of Ni(II)/Zn(II), Ni(II)/Cu(II) and Ni(II)/Co(II) from their binary mixture was also investigated by batch procedure. These ions have the same charge and similar ionic radius<sup>6</sup>. In their binary mixture the two metal ions had the same concentration of  $10 \mu\text{g mL}^{-1}$  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 IIP for Ni(II) was large, while decreased significantly for Co(II), Cu(II) and Zn(II). The relative selectivity factor ( $\alpha_r$ ) values of Ni(II)/Co(II), Ni(II)/Cu(II) and Ni(II)/Zn(II) were 374.0, 294.0 and 351.1, respectively, which are more than 1 (Table-1). This means that Ni(II)-imprinted sorbent had higher selectivity for Ni(II). And the results indicated that Ni(II) could be determined even in the presence of Co(II), Cu(II) and Zn(II) interferences.

The effect of different foreign ions, KCl,  $\text{MgSO}_4$ ,  $\text{NaNO}_3$ ,  $\text{KNO}_3$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{Na}_3\text{PO}_4$ , KI and  $\text{CaCl}_2$ , on the adsorption of Ni(II) was studied using the batch procedure. The results showed that 60-300-fold electrolytes did not interfere with adsorption and desorption of Ni(II), indicating that the present imprinted polymers can be suitably used as sorbents for Ni(II)

Metal ions	E (%) (IIP)	E (%) (NIP)	$D_i$ ( $\text{mL g}^{-1}$ )	$D_n$ ( $\text{mL g}^{-1}$ )	$\alpha_i$	$\alpha_n$	$\alpha_r$
Ni	99.0	15.0	19800	35.3	—	—	—
Co	38.1	28.8	123.1	80.9	160.8	0.43	374.0
Cu	16.2	9.2	38.7	20.3	511.6	1.74	294.0
Zn	10.0	6.5	22.2	13.9	891.9	2.54	351.1

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 Ni ions was examined under the optimum conditions (pH, eluent, etc.). A volume of 50 mL of the sample containing  $40 \mu\text{g L}^{-1}$  of Ni ions was passed through the IIP column. The flow rate was adjusted in the range of  $0.5\text{-}2.0 \text{ mL min}^{-1}$  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  $2.0 \text{ mL min}^{-1}$ . Thus, a flow rate of  $2.0 \text{ mL min}^{-1}$  was selected in this work.

For the elution of Ni(II) from the column, three parameters, *i.e.*, elution volume, eluent ( $\text{HNO}_3$ ) flow rate and eluent concentration were investigated and optimized. Flow rate of  $\text{HNO}_3$  solution was the first parameter to be optimized. A volume of 50 mL of the sample containing  $40 \mu\text{g L}^{-1}$  of Ni ions was passed through the IIP column at  $2.0 \text{ mL min}^{-1}$ . Elution of the SPE column was performed with 5 mL of 1 M  $\text{HNO}_3$  at different flow rates between  $0.1$  and  $0.8 \text{ mL min}^{-1}$ . The results showed that 5 mL of 1 M  $\text{HNO}_3$  at a flow rates of  $0.2 \text{ mL min}^{-1}$  was sufficient for 95% recovery. In order to find optimum volume of the elution solvent, different volumes of  $\text{HNO}_3$  from 2-7 mL were investigated. The results are displayed in Fig. 3. As can be seen from the figure, quantitative elution was attained using volumes  $\geq 5$  mL. The influence of  $\text{HNO}_3$  concentration on the elution of Ni ions from the sorbent was also examined and the results are shown in Fig. 4. Nickel ions were quantitatively eluted from the column with 1 M  $\text{HNO}_3$  solution.

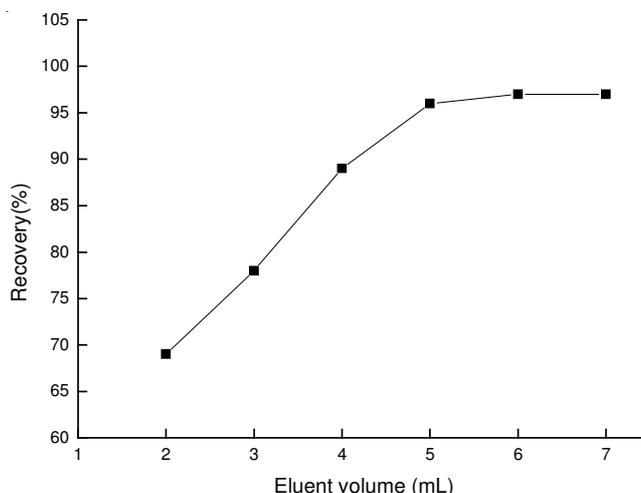


Fig. 3. Influence of eluent volume on the elution of Ni(II) from the Ni(II)-IIP. Sample volume: 50 mL, sample pH 7, Ni(II) concentration:  $40 \mu\text{g L}^{-1}$ ,  $\text{HNO}_3$  flow rate:  $0.2 \text{ mL min}^{-1}$ ,  $\text{HNO}_3$  concentration: 1 M

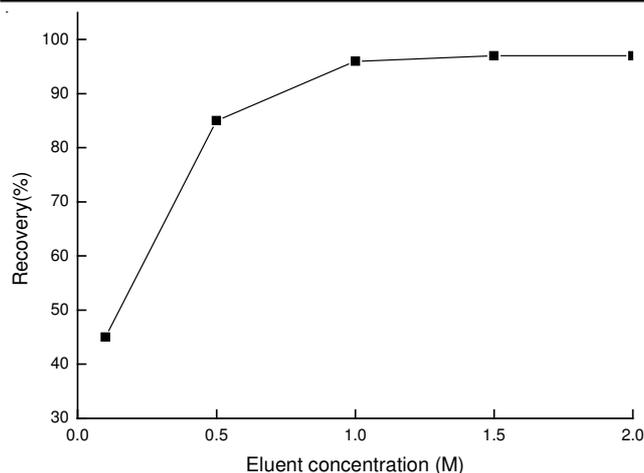


Fig. 4. Influence of  $\text{HNO}_3$  concentration on the elution of Ni(II) from the Ni(II)-IIP. Sample volume: 50 mL, sample pH 7, Ni(II) concentration:  $40 \mu\text{g L}^{-1}$ ,  $\text{HNO}_3$  flow rate:  $0.2 \text{ mL min}^{-1}$ , elution volume: 5 mL

The capacity of imprinted polymers had no considerable decrease after 50 cycles of repeated experiments. The Ni(II)-IIP showed good reusability and stability towards Ni(II).

**Maximum sample volume and enrichment factor:** The enrichment factor was studied following recommended column procedure by increasing volume of Ni(II) solution and keeping the total amount of loaded Ni(II) constant to  $2.0 \mu\text{g}$ . For this purpose, 50, 100, 150, 200, 250, 300 and 350 mL of sample solutions containing  $2.0 \mu\text{g}$  Ni(II) were passed through the column at the optimum flow rate. The results showed that the maximum sample volume could be up to 250 mL with the recovery  $> 95\%$ . Therefore, 250 mL of sample solution was adopted for the preconcentration of analytes from sample solutions. And a high enrichment factor of 50 was obtained.

**Analytical accuracy and precision of IIPs-SPE:** Under the selected conditions, eleven portions of  $5.0 \mu\text{g L}^{-1}$  Ni(II) standard solutions (250 mL) were treated and analyzed simultaneously following the column procedure. The relative standard deviations of the method was  $2.5\%$ , indicating that the method had good precision for the analysis of trace Ni(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  $1.0 \text{ M}$  nitric acid calibration graph<sup>4</sup>, was calculated to be  $0.2 \mu\text{g L}^{-1}$  for a pre-concentration factor of 50.

The method has been applied to the determination of Ni(II) in biological standard reference materials (GBW10016, Tea). The results are given in Table-2.

Sample	Certified value ( $\mu\text{g g}^{-1}$ )	Present value ( $\mu\text{g g}^{-1}$ )
Tea (GBW10016)	$3.4 \pm 0.3$	$3.5 \pm 0.4$

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 nickel were in the range of 96.5-103.5%. These results indicated the suitability of the Ni(II)-IIP for selective solid-phase extraction and determination of trace Ni(II) from biological samples.

Sample	Added ( $\mu\text{g g}^{-1}$ )	Found by present method ( $\mu\text{g g}^{-1}$ )	Recovery (%)
Pinus leaves	0	$2.8 \pm 0.2$	–
	20	$23.0 \pm 0.3$	101
Rice	0	$2.5 \pm 0.2$	–
	20	$21.8 \pm 0.4$	96.5
Human hair	0	$4.8 \pm 0.3$	–
	20	$25.5 \pm 0.2$	103.5

–: Not determined.

## Conclusion

A new Ni-imprinted sorbent based on 5-(4-carboxyl-phenylazo)-8-hydroxyquinoline ligand was developed as solid phase extraction material for selective preconcentration of Ni ions from biological samples. The imprinted polymer has obvious imprinting effect on the imprinting ion. The values of the relative selectivity factor were found to be 351.1, 294.0 and 374.0 for Zn(II), Cu(II) and Co(II), respectively. The method shows good linearity and reproducibility. The proposed procedure was applied for the determination of Ni in biological samples.

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