



## Determination of Cobalt and Nickel in Urine Samples Based on Modified Magnetite Nanoparticles as Adsorbents

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The magnetic solid-phase extraction, based on the adsorption of 1-(2-pyridylazo)-2-naphthol as ions exchange groups onto magnetite nanoparticles ( $\text{Fe}_3\text{O}_4$  nanoparticles) to form modified magnetic nanoparticles, was proposed for the preconcentration of cobalt and nickel from urine samples by graphite furnace atomic absorption spectrometry (GFAAS). Various parameters affecting the adsorption of cobalt and nickel were investigated, such as pH of the solution, type, volume and concentration of desorbing reagent, amount of adsorbent and interference effects. Under the optimum conditions, the calibration graphs were linear in the range of 10-50 and 10-60  $\text{ng L}^{-1}$  with detection limit of 2.5 and 3  $\text{ng L}^{-1}$  for cobalt and nickel, respectively. A good relative standard deviation for 10 determination of 20  $\text{ng L}^{-1}$  of cobalt and nickel were 3.85 and 4.25 %, respectively. The proposed method was applied to the analysis of four urine samples with satisfactory results.

**Keywords:** Magnetic nanoparticles, 1-(2-Pyridylazo)-2-naphthol, Magnetic solid-phase extraction, Cobalt, Nickel.

### INTRODUCTION

Nowadays, magnetic solid-phase extraction (MSPE) has attracted much interest in sample preparation<sup>1-3</sup>. A distinctive superiority of this technique is that magnetic materials can be readily isolated from sample solutions by the application of an external magnetic field. Moreover, these particles possess excellent magnetic responsivity, high dispersibility and easiness of surface modification<sup>4</sup>.

All of these merits render magnetic solid-phase extraction a promising technique for direct analysis of samples containing particles or microorganisms, which are widely existing in environmental or biological matrices and may arouse blockage and lead to extraction failure on conventional solid phase extraction cartridges<sup>5</sup>. Magnetic solid-phase extraction have been successfully applied to the separation and enrichment of organic and inorganic contaminants from environmental samples<sup>6-10</sup>. Hsu and Whang<sup>6</sup> synthesized functionalized magnetic nanoparticulate adsorbents for rapid extraction of glyphosate and its major metabolite aminomethyl-phosphonic acid from water samples. Faraji *et al.*<sup>7</sup> utilized decanoic acid-coated  $\text{Fe}_3\text{O}_4$  nanoparticles as an adsorbent to extract some heavy metal ions in water samples. However, the magnetic adsorbents in these studies were mainly applied to the analysis of water samples, studies on the application in urine samples were hardly reported.

This study proposes a preconcentration procedure using modified magnetic nanoparticles with 1-(2-pyridylazo)-2-naphthol (PAN) based on simple physical adsorption for the determination of cobalt and nickel in urine samples by graphite furnace atomic absorption spectrometry (GFAAS). The aim of this study is to establish a preconcentration method in urine samples, which can not only isolate the analytes from environmental matrices, but also reduce the matrix effects effectively.

### EXPERIMENTAL

The experiments were performed using a Shimadzu (Kyoto, Japan) atomic absorption spectrophotometer (AA6300G) with a graphite furnace atomizer (GFA-EX7i). The instrumental parameters were adjusted according to the manufacturer's recommendations. A pH3-3C digital pH meter equipped with a combined glass-calomel electrode (Hangzhou Dongxing Instrument Factory, Hangzhou, China) was used for pH adjustment. A 59 kHz, 200 W ultrasonic bath with temperature control (Shanghai Kudos Ultrasonic instrument Co, Ltd., Shanghai, P.R.China) was used to mix the sorbent with the sample.

$\text{Fe}_3\text{O}_4$  nanoparticles were prepared by chemical coprecipitation method<sup>11</sup>. The fabrication process of modified magnetic nanoparticles (designated  $\text{Fe}_3\text{O}_4@PAN$ ) was similar to Khajeh and Sanchooli<sup>12</sup>. An ethanolic solution of 1-(2-pyridylazo)-2-

naphthol (PAN) was added to an appropriate amount of dried  $\text{Fe}_3\text{O}_4$  nanoparticles in a beaker. The mixture was gently stirred and solvent was completely eliminated by heating the modified particles in 20 min at 60 °C.

The magnetic solid-phase extraction procedure was carried out as follows: 4.5 mg  $\text{Fe}_3\text{O}_4$ @PAN nanoparticles were added to the 50 mL filtered urine sample or standard solution. The pH was adjusted to 5 with ammonium acetate buffers solution and the mixture was sonicated for 2 min. After equilibrium, an Nd-Fe-B strong magnet was deposited at the bottom of the bottle and the sorbents were isolated from the solution. After about 1 min, the solution became clear and the supernatant was decanted. Finally, 2 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub> (containing 10 % ethanol) was added to desorb the analytes (washed twice, 1 mL each time). The eluent was collected. After filtration through 0.45 μm membrane, 20 μL eluent was removed using a 50 μL microsyringe and injected into the GFAAS for analysis.

## RESULTS AND DISCUSSION

The effect of pH on the solid phase extraction of cobalt and nickel was investigated because this parameter plays an important role in metal-chelate formation. For this study, a pH range of 2-10 was used. The results are shown in Fig. 1. It can be seen that extraction was quantitative for cobalt in the pH range 4-6. For nickel, the quantitative extraction was achieved when the pH exceed 5. These are coincident with the optimal pH range of chelate formation between cobalt and nickel and 1-(2-pyridylazo)-2-naphthol. Hence, a pH of 5 was chosen for the subsequent work.

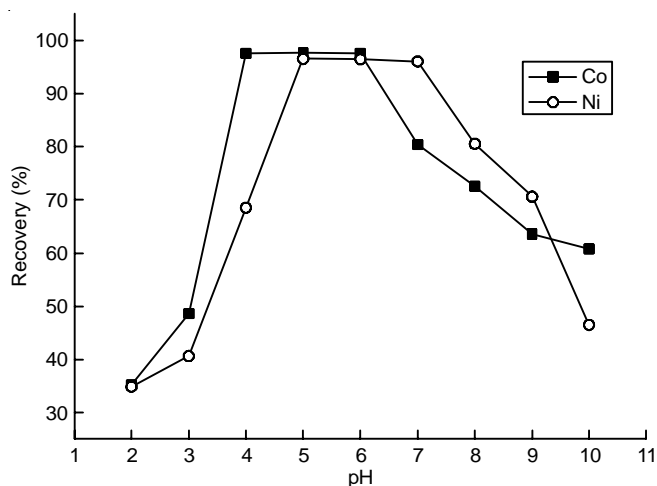


Fig. 1. Effect of pH on the recoveries of analytes obtained from MSPE-GFAAS. Extraction conditions: water sample volume, 50 mL; eluent type, 1 mol L<sup>-1</sup> HNO<sub>3</sub> (containing 10 % ethanol); eluent volume, 2 mL

In the present work, the  $\text{Fe}_3\text{O}_4$ @PAN showed an excellent capability to retain the heavy metal ions. The influence of the mass of modified nanoparticles (1-5 mg) used for quantitative extraction of cobalt and nickel were investigated. Optimum amount of modified nanoparticles was 4.5 mg. This can be attributed to the fact that as the mass of nanoparticles increased, the amount of 1-(2-pyridylazo)-2-naphthol increased, therefore, was increasing the percent of metal ions recovery. Subsequent experiments were performed for 4.5 mg  $\text{Fe}_3\text{O}_4$ @PAN

(the ratio of  $\text{Fe}_3\text{O}_4$  nanoparticles and 1-(2-pyridylazo)-2-naphthol was kept at a constant 2:1).

Nitric acid was known to disrupt metal ions and was preferable in GFAAS measurements due to the better repetitively. Desorption ability of HNO<sub>3</sub> containing 10 % ethanol was found to be superior to that without adding ethanol. The effect of eluent volume on the recoveries of the analytes was also studied by using 1 mol L<sup>-1</sup> HNO<sub>3</sub> in ethanol. It was found that quantitative recoveries could be obtained with 2-4 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub> in ethanol. Therefore, the volume of 2 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub> (containing 10 % ethanol) was used in the following experiments.

Effect of foreign ions on the preconcentration and determination of cobalt and nickel was investigated. A given spices was considered to interfere if it resulted in a  $\pm 5$  % variation of the GFAAS signal. The effect of different ions on simultaneous determination of 20 ng L<sup>-1</sup> cobalt and nickel standard solution was studied. Among the ions tested Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Pb<sup>2+</sup>, Mn<sup>2+</sup>, Cd<sup>2+</sup>, Al<sup>3+</sup>, Cr<sup>3+</sup>, Ag<sup>+</sup>, F<sup>-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, CO<sub>3</sub><sup>2-</sup>, CH<sub>3</sub>COO<sup>-</sup> did not show interferences at the concentration of 500 times higher than that of cobalt and nickel concentration, however Cu<sup>2+</sup> and Zn<sup>2+</sup> showed interference at the concentration 20 times higher than that of analytes. The interference of Cu<sup>2+</sup> and Zn<sup>2+</sup> were eliminated upon addition of EDTA (added after complex formation procedure) at a concentration of 1 × 10<sup>-3</sup> mol L<sup>-1</sup>. These results indicate that the developed method is applicable to analysis of cobalt and nickel in urine samples.

The analytical characteristics of the developed method, including linear range, the limit of detection, precision and regression equation were obtained by processing standard solution of cobalt and nickel (Table-1). For a sample consumption 50 mL, the detection limit, defined as 3Sb/m (where Sb and m are standard deviation of the blank and slope of the calibration graph, respectively), was 2.5 and 3 μg L<sup>-1</sup> while the precision (RSD) was 3.85 and 4.25 % for cobalt and nickel, respectively.

Analytical parameters	Co	Ni
Linear range (ng L <sup>-1</sup> )	10.0-50.0	10.0-60.0
Regression equation ([M] in ng L <sup>-1</sup> )	$3 \times 10^{-3}C + 0.0026$	$2.8 \times 10^{-3}C + 0.0045$
Correlation coefficient (R <sup>2</sup> )	0.9993	0.9991
Limit of detection (ng L <sup>-1</sup> )	2.5	3.0
Precision (R.S.D. %) (n = 10, 20.0 ng L <sup>-1</sup> )	3.85	4.25
Sample consumption (mL)	50.0	50.0

The proposed method was applied to the determination of cobalt and nickel in urine samples. Because certified reference materials for urine were not available, the validation of the method was performed by the analysis of spiked samples. Table-2 showed that the proposed method can be successfully applied to the determination of cobalt and nickel in urine samples.

A preconcentration procedure using modified magnetic nanoparticles with 1-(2-pyridylazo)-2-naphthol based on

TABLE-2  
ANALYTICAL RESULTS OF CO AND Ni  
DETERMINATION IN SPIKED URINE SAMPLES  
WITH THE MSPE-GFAAS METHOD (n=3)

Sample	Spiked (ng L <sup>-1</sup> )		Found (ng L <sup>-1</sup> ) <sup>a</sup>		Recovery (%)	
	Co	Ni	Co	Ni	Co	Ni
1	0.0	0.0	3.60±0.21	ND <sup>b</sup>	–	–
	10.0	10.0	13.35±0.60	9.54±0.45	97.5	95.4
	15.0	15.0	18.36±0.75	14.43±0.65	98.4	96.2
2	0.0	0.0	4.50±0.25	3.20±0.18	–	–
	10.0	10.0	14.28±0.60	12.92±0.54	97.8	97.2
	15.0	15.0	19.29±0.78	17.87±0.75	98.6	97.8
3	0.0	0.0	ND	2.70±0.15	–	–
	10.0	10.0	9.56±0.45	12.40±0.52	95.6	97.0
	15.0	15.0	14.46±0.64	17.32±0.72	96.4	97.5
4	0.0	0.0	5.40±0.32	ND	–	–
	10.0	10.0	15.22±0.68	9.52±0.42	98.2	95.2
	15.0	15.0	20.32±0.81	14.48±0.64	99.5	96.5

<sup>a</sup>Mean of three experiments ± standard deviation; <sup>b</sup>Non detected

simple physical adsorption was successfully applied for the determination of cobalt and nickel by GFAAS. This method is rapid, easy, safe and inexpensive for determining trace cobalt and nickel in urine samples. Compared to traditional solid phase extraction method, it avoids the time-consuming column passing or filtration operation and shows great analytical potential to separate and preconcentrate target analytes from complex samples without clean-up steps.

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