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ORIGINAL ARTICLE

Comparative Study of Nickel Recoveries from Urine, Nail, and Hair Samples Using XAD-7 and Chromosorb 105 Resins

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ABSTRACT

Nickel is widely used in many industries. It is known as an immunotoxic, neurotoxic, pulmonary toxic, nephrotoxic, hepatotoxic, and carcinogenic agent. Biological monitoring is a useful tool to assess the internal exposure to toxic compounds. Because of trace concentration of analytes in biological samples and matrix interferences, the preparation of such complicated samples is necessary prior to analysis. This study aimed to optimize the parameters affecting solid phase extraction (SPE) procedure and compare the enrichment recoveries of sorbents XAD-7 and chromosorb 105 for preconcentration and separation of nickel in urine, hair and nail samples. Solid phase extraction using both resins was optimized regarding the sample pH, ligand concentration, loading flow rate, elution solvent, sample volume, elution volume, amount of resins, and matrix interferences. The optimized procedure was validated based on the “within-day” and “day-to-day” reproducibility experiments. The proposed method was applied for pre-treatment of urine, hair and nail samples. Nickel was extracted with recoveries in the range of 94-100%. A good reproducibility and repeatability was obtained for optimized method using both resins under the existing conditions. The concentration of nickel in workers’ biological samples was determined using two sorbents. There was no significant difference between obtained recoveries for XAD-7 and chromosorb 105. This optimized method can be successful in simplifying sample preparation for trace residue analysis of Ni in different matrices for evaluation of occupational and environmental exposures. Resin XAD-7 is recommended for next experiments because of its lower cost and simpler preconditioning compared to chromosorb 105.

Keywords: *Solid phase extraction, Sample Preparation, Heavy Metals, Urine, Nail, Hair*

INTRODUCTION

Heavy metals can be considered as a unique class of environmental toxicant. Nickel, as a heavy metal, is used in various processes such as electroplating, alloy production, and nickel-cadmium batteries. Exposure to nickel is common in industries where the metals are used in a wide range. The presence of nickel may cause functional impairments by competition with binding sites in biological systems [1, 2]. Nickel is known as an immunotoxic, neurotoxic, pulmonary toxic, nephrotoxic, hepatotoxic, and carcinogenic agent. It is

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an allergen agent, causes skin reaction in humans sensitive to nickel. International Agency for Research on Cancer has classified the nickel compounds as human carcinogens [3].

Because the usage of nickel is unavoidable, study of this compound is of great interest from the occupational viewpoints. On the other hand, there are major problems in heavy metals determination stages. Therefore, sensitive and accurate techniques to determine metal ions at trace levels in different samples like water and biological fluids are required in environmental and occupational health studies [4, 5].

In the analysis of trace elements, there is a tendency to measure accurately low contents of analytes. Although the modern equipments have improved the selectivity of the analytical procedures, they are expensive and maybe unavailable in most laboratories. Consequently, sample preparation procedures, which can be performed in any laboratory, have been developed to simplify analytical approaches, leading to a reduction in time, expense and personnel required to carry out the analytical procedure. The selection of a procedure at which analytes can precisely be detected or determined is important [6, 7]. To extract heavy metals, many sample preparation procedures are being used such as soxhlet extraction [8], liquid-liquid extraction [9-11], supercritical fluid extraction (SFE) [12], and solid phase extraction [8, 13-15]. Soxhlet and LLE are time-consuming procedures and the recoveries obtained from such methods are not often suitable and reproducible. Therefore, more sensitive and precise methods are required to measure trace heavy metals in biological and environmental samples.

Solid phase extraction has been considered as a separation/preconcentration method because of its advantages. It is a simple and rapid method in which a high preconcentration factor is obtained. Through SPE procedure, separation and purification of the compound of interest can be achieved and only low volumes of solvents are used. SPE is an appropriate procedure to preconcentrate trace heavy metal ions from various media such as seawater, mineral waters, urine and other environmental and biological samples [2, 16-17]. In extraction process, various sorbents such as activated carbon, C18, chelating resins, silica gel and recently carbon nanotubes can be used [14, 18-19]. These sorbents can be screened and selected, depending on the chemical nature of the analyte. In this regard, amberlite XAD polymeric adsorbents are very porous polymers with uniform pore size distribution, that their high internal surface areas can adsorb a wide variety of different species. They are stable in a wide range of pH. These resins are synthesized by cross-linking reaction between styrene and divinylbenzene [20]. Besdies, chromosorb resins are synthetic polymers with good physical and chemical properties such as porosity, high surface area, durability, and high stability over the entire pH range [9, 20].

Human biomonitoring has being used in occupational health as a useful tool to assess low levels of chemicals. In this respect, the choice of the most appropriate biological samples is important. Although blood and urine samples are the most widely used matrices for biomonitoring chemicals such as heavy metals in occupational and environmental toxicology, the development of new methodology allows the use of more matrices like saliva, nails, hair, and breast milk. Human hair and nail have advantages over other biological samples; they are stable matrices, their collection and transportation are simpler and do not change over the period between sampling and analysis. In addition, urine is a preferred matrix in heavy metals biomonitoring and it can be collected in larger amounts when it is needed [21-23].

This study was aimed to compare the efficiency of resins XAD-7 and chromosorb 105 and achieve optimum criteria necessary for development of a sample preparation procedure for nickel, present in urine, hair, and nail samples, leading to a simple protocol of SPE method in environmental and occupational exposures.

MATERIAL AND METHODS

Chemicals and reagents

All solutions were prepared using distilled water. Nickel stock solution was prepared from appropriate amount of its nitrate salt (Merck, Darmstadt, Germany) as 1000 mg/lit solution in 0.01M HNO_3. Working and standard solutions were prepared daily by dilution of the stock solution. Acids and other chemicals used in this study were obtained from Merck, Darmstadt, Germany. Standard buffered solution at various pH values, amberlite XAD-7 resin (20-40 mesh), and Ammonium Pirrolidine Dithio Carbamate (APDC) were also purchased from Merck. Chromosorb 105 resin (80-100 mesh) was obtained from Sigma-Aldrich.

Apparatus

Polarograohic determination of nickel was done by Metrohm 757 Computrace VA voltameter. The pH values of the solutions were measured by a digital pH meter model Metrohm 744. Amount of reagents were measured using a Satorius CP225D balance (Sartorius, Germany).

Mini columns preparation

Glass mini columns (100 × 10 mm) were packed with 500 mg resin, separately for each resin. After packing, a little amount of glass wool was placed at both ends of the glass tube. Before using the column, amberlite XAD-7 resin was washed by ethanol, water, 1M HCl, water, respectively. Methanol, water, 1 M HNO3, water, 1 M NaOH and water were used to wash Chromosorb 105, respectively. Finally, resins were pre-concentrated with buffer solution.

Table 1. Effect of sample pH, ligand concentration, eluent type, and eluent volume on recovery of Ni (II) from XAD-7 and Chromosorb-105 resins (eluent: 2M HNO3)

	M (%)±SD			M (%)±SD			M (%)±SD			M (%)±SD	
pH	XAD-7	Chr.105	Ligand Concentration w/v (%)	XAD-7	Chr.105	Eluent type	XAD-7	Chr.105	Eluent Volume (ml)	XAD-7	Chr.105
2	10±0.00	10±0.00	0.01	32±4.47	54±5.47	HCl	28±4.47	92±4.47	5	48±1.12	46±2.23
4	16±5.47	12±4.47	0.03	76±5.47	90±0.00	Acetone	94±5.47	72±4.46	10	62±2.73	83±2.73
7	50±0.00	60±0.00	0.05	98±4.47	98±8.36	(HNO_3 in Acetone)	97±8.36	90±0.00	15	95±4.21	97±4.21
9	98±4.47	98±4.47	0.07	96±5.47	94±5.47	1M HNO_3	95±5.47	96±5.47	20	98±4.47	96±5.47
						2M HNO_3	96±5.47	97±5.47			

Pre-concentration procedure

Solid phase extraction using XAD-7 and chromosorb 105 resins was optimized with regard to 9 following parameters: sample pH, sample and eluent flow rates, elution solvent, eluent volume, ligand concentration, amount of resin, and sample volume. Fifty milliliter solution containing 20 µg of Ni (II), 10 ml buffer solution with desired pH and 6 ml APDC solution (as ligand) was prepared. Samples were then passed through the at a flow rate of 5 ml/min. The column was then washed with 5-10 ml of the same buffer solution. Therefore, the metal ions were eluted from the mini column with 10-15 ml of different solvents. Eventually, the nickel concentration in the solution was determined by polarographic technique. All steps were performed separately for each resin.

Collection and Pre-treatment of urine, hair, and nail samples

Biological samples were collected from 15 subjects who work in metal industries. After obtaining approval from Tehran University of Medical Sciences (Vice-Chancellor for Research), all participants signed related consent form for the voluntary participation. Then, their urine, hair, and nail samples were collected in the end of shift under documented consent and stored at proper conditions until use.

The weighted, cleaned, and dried hair and nail samples were separately placed into labeled beakers. After that, acid digestion was done based on recommended method [24-26] until samples dissolved, obtaining clear solutions. After diluting the urine samples, for adjusting their pH near the pH 9, sodium hydroxide solution was used. For all pre-treated biological samples, aforesaid pre-concentration procedure was performed.

RESULTS

Effects of different parameters on extraction recovery

To examine the effects of different parameters on sorption and extraction recoveries of nickel, pre-concentration procedure was performed by both resins to optimize mentioned parameters.

The effect of each parameter was separately examined in several levels, then, the optimized factor was selected based on obtained recoveries. After selecting the optimum value for a parameter, it was used in all next experiments. Table 1 and 2 indicate the levels of all parameters and related recoveries for resins XAD-7 and chromosorb 105. The optimum values for examined parameters were pH of 9, ligand concentration: 0.05%, loading flow rate: 5 ml/min, elution solvent: 2M HNO_3, sample volume up to 500 ml, elution volume: 15 ml, and amount of resins: 500 mg. The effect of various matrix ions mostly present in environmental and biological samples, including Na^+, K^+, Mg^{2+}, Ca^{2+}, and SO_4^{2-} was examined as another parameter. Quantitative recoveries for 20g/l of NaCl, 1g/l of K^+, 1g/l of Mg^+, for 1g/l of Ca^{2+}, and 1.5g/l of $SO_2^{-\ 4}$ were obtained. The results clearly showed the non-effectiveness of all added components on the recoveries obtained from optimized method. Using the presented procedure, nickel was extracted with recoveries in the range of 94-100%. Both resins indicated high enrichment recoveries for nickel ions and there was no significant difference between two solid phases ($p > 0.05$).

Reproducibility

The reproducibility of the method was evaluated by three spiked samples at concentrations of 1, 1.5, and 2 µg/ml under the optimized conditions. Related experiments were followed by analysis of samples, each day for 6 consecutive days to estimate day-to-day reproducibility as well as six times a day to estimate

Table 2. Effect of eluent flow rate, sample flow rate, sample volume, and sorbent mass on recovery of Ni (II) from XAD-7 and Chromosorb-105 resins (eluent: 2M HNO3)

	M (%)±SD			M (%)±SD			M (%)±SD			M (%)±SD	
Eluent flow rate (ml/min)	**XAD-7**	**Chr.105**	**Sample flow rate (ml/min)**	**XAD-7**	**Chr.105**	**Sample volume (ml)**	**XAD-7**	**Chr.105**	**Sorbent amount (mg)**	**XAD-7**	**Chr.105**
2	98±4.47	98±8.36	2	96±0.00	98±4.47	50	98±4.47	96±5.47	100	50±0.00	54±5.44
5	98±4.47	98±5.47	5	96±4.47	96±5.47	150	98±4.47	96±5.47			
7	94±5.47	96±4.47	7	94±4.47	94±5.47	250	96±5.47	96±5.47	500	98±4.47	98±4.47
10	82±4.47	92±5.47	9	94±4.47	90±0.00	500	96±5.47	94±5.47			
						750	64±5.47	82±4.47			

within-day reproducibility. In within-day experiments evaluation, one urine samples per day were analyzed six times for three consecutive days. In day-to-day reproducibility, experiments were performed every day for 6 consecutive days with three replications. Linear standard curves (extracted) over the concentration ranges were obtained with a correlation coefficient of 0.991 or greater. Coefficient of variations (CV %) related to both resins showed suitable accuracy and precision (Table 3).

Analyte ions in real samples

The preconcentration method was applied to the polarographic determination of nickel in urine, hair, and nail samples collected from subjects who were working in metal industries. Table 4 indicates the results related to both resins.

DISSCUSION

The results showed that, efficient recoveries have been obtained from XAD-7 and Chr-105 resins using sample pH of 9. It seems that, at this pH level, the analyte of interest is mostly in the ionized form, making it to be easily retained on the ionized ligand already conjugated to the sorbents. Sample pH of 9 was selected as the confident value for further study (Table 1).

Since there is no affinity between non-polar sorbents and the ionized analyte, a conjugating ionized ligand was used on the sorbents to follow up an ionized extraction mechanism. APDC showed to be an appropriate ligand for capturing nickel (II) from the sample, however, from the four concentrations of the ligand, the amounts of 0.05, and 0.07 (%) indicated good efficiencies for retaining of the analyte. To prevent the saturation of sorbents with the ligand and reduce the reagents through extraction process, the amount of 0.05% was selected as the optimum level for both sorbents (Table 1).

Considering the properties of analytes both ionizability and hydrophobicity were useful to obtain efficient extraction recovery. Highly ionic compounds can strongly retain on ionic conjugated sorbents, leading to difficult elution and subsequent poor recoveries. From the eluents used in this study, as the Table 1 shows, the HNO_3 based solutions were more efficient for both sorbents, because they were organic free eluents and can prevent co-elution of organic compounds possibly present in the real samples as well as reducing exposure to such evaporative and hazardous solvents. The maximum recovery has been achieved using 2 M HNO_3 and, therefore, it was selected as the optimum eluent.

According Table 1, the eluent volumes 15 and 20 ml indicated the highest recoveries of nickel desorption from XAD-7 and Chr-105, respectively. Since there was no significant difference between 15 and 20 ml for XAD-7 ($p > 0.05$), the volume of 15 ml was selected for next experiments to achieve the higher concentration factor. The volume of eluent depends on two important parameters; the strength of eluent retention and the sorbent mass used in SPE.

The greater the elution strength, the less volume the eluent. In addition, using a larger sorbent mass cartridge leads to increase the elution volume. The lowest satisfactory eluent volume in this study (15 ml) resulted in a suitable concentration factor of 33.3.

In addition to the eluent volume, the faster elution can affect on the whole analysis time when numerous samples is going to be applied. Therefore, among the examined levels, the eluent flow rate of 5 ml/min was selected as the optimum level to reduce the elution time (Table 2).

Table 3. Day-to-day (D-day) and within-day (W-day) reproducibility of Ni (II) spiked in urine for XAD-7 and Chromosorb-105 resins (sample volume: 50 ml, N=6)

Sorbent	Statistical data	Concentration added (µg/l)					
		1		1.5		2	
		D-day	W-day	D-day	W-day	D-day	W-day
XAD-7	Mean	0.966	0.970	1.440	1.450	1.920	1.930
	SD	1.633	1.095	1.673	0.816	1.095	1.032
	CV%	1.69	1.13	1.74	0.84	1.14	1.032
Chr.105	Mean	0.97	0.96	1.45	1.449	1.94	1.96
	SD	1.61	1.06	1.505	1.52	1.094	1.093
	CV%	1.65	1.67	1.55	1.61	1.08	1.15

Table 4. Nickel concentration in urine, hair, and nail samples of workers (N=15)

Sorbent	Nickel Concentration		
	Urine (µg/l) Mean ± SD	**Hair** (µg/g) Mean ± SD	**Nail** (µg/g) Mean ± SD
XAD-7	539±230	847±280.6	1539±230.1
Chr.105	595±183	979±301.2	1725±301.5

The experiment on sample volume allowed an accurate measurement as low as 0.04 µg/ml of nickel when a large sample volume (500 ml) is applied on the column. Therefore, trace enrichment of the analyte with an appropriate concentration factor of 33.3 was obtained that can be compatible to current analysis systems in most laboratories.

In this study, sample flow rate up to 9 ml/min were applied with acceptable recoveries of 94 and 90% for XAD-7 and Chr-105, respectively (see Table 2). Therefore, to be confident, the sample flow rate of 5 ml/min was selected for both sorbents (96% recovery). However, based on results, it would be possible to increase the sample flow rate without significant loss in the analyte recovery.

The obtained results indicated that, the sorbents amount of 100 mg was not appropriate amount, because the breakthrough happened through the experiments. By using the sorbent mass as large as 500 mg, taking place the longer interaction caused retention of significant amount of nickel on the sorbents and subsequent efficient recoveries of 98% (Table 2).

In order to examine the effect of possible matrix components on the optimized method, three different concentrations of similar ions were added to the sample. The added ions were chosen based on the possible interferences presenting in the real environmental samples and matrices. As it was stated in the results, the non-effectiveness of all added components for each concentration on the recoveries was obtained from optimized method. The high recoveries indicated that no cross-reactivity is taken place between added interferences and both sorbents and no co-elution is happened.

Finally, in order to validate the method, day-to-day and within-day reproducibility of the optimized method were determined by three spiked extracted samples according to the procedure explained in material and methods. A linear standard curve (for extracted sample) was obtained with the correlation coefficient of 0.995 or greater. Table 3 indicates coefficient of variations (CV%) and standard deviations (SD) for each concentration, confirming the suitable accuracy and precision.

Comparatively, using solid phase extraction may overcome some problems with other methods like liquid-liquid extraction (LLE). There are still no basic rules for selection of a solvent system for extraction of analytes by LLE and selection of a solvent is still empirical. The use of large volumes of toxic solvents, contamination of extracts from solvents and glassware, and low recovery are other problems associated with LLE. Therefore, nowadays, there is a strong trend towards replacing LLE by SPE. According to literatures [14, 15, 27], authors generally have used 5-6 factors to optimize the SPE method, while, in this study, nine parameters were screened optimized, in which a robust and more reliable method is introduced. Moreover, compared to the other studies [14, 20, 28], in this study, further experiments of reproducibility were done using spiked urine samples. Finally, the validated optimized method was used for biological monitoring of worker exposed to nickel. Biological samples like hair and nails contain trace amounts of analytes and there are interfering compounds in such matrices, therefore, SPE methods can be useful and suitable for their purification and concentration. There are a few studies on the biological monitoring of toxic compounds in workplaces. Kargar et al. used the SPE method to preconcentrate urine samples for evaluating the occupational exposure of glazers to cobalt in a ceramic industry [27]. As it was mentioned in results, the optimized method in present study was used to prepare the urine, hair, and nail samples for evaluating the workers exposure to nickel ions. Other investigations on hair and nail samples have not used the preparation methods like SPE [29-31]. They have determined the low-level concentration of analyte using modern analytical systems. Soylak et al. used SPE procedure successfully and efficiently for separation and preconcentration of nickel, lead, and copper in urine samples [2].

Although, both studied resins showed high and considerable recoveries to extract the nickel ions and there was no significant difference between them in all experiments ($p > 0.05$), XAD-7 was introduced for evaluation the exposure of workers for next studies because of two important reasons. Firstly, the resin XAD-7 is inexpensive sorbent (its cost is nearly one tenth of chromosorb 105) and secondly, the pre conditioning of XAD-7 like providing its paste to place into glass columns is much easier than the chromosorb resins.

CONCLUSIONS

This study confirmed that, SPE is an efficient sample preparation procedure, especially for biological matrices, and has more advantages than the liquid-liquid extraction. Depending on the chemical and physical properties of the analyte, a wide range of sorbents is available. In addition, the optimized method provides the reliable, easy to use, and cost effective procedure to overcome difficulties associated with other sample preparation techniques. The concentration factor for both resins was 33.3 and both of them can be used several times. Although, both resins are the same in respect of extraction recoveries and other properties, the resin cost is an important reason to choose XAD-7 for next studies or evaluation programs. The optimized method is promising to be used as a simple and applicable procedure for pre-concentration of other metal ions when analysis of trace heavy metals in biological and environmental samples is of interests.

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The authors declare that there is no conflict of interests.

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