



Determination of Some Heavy Metals in Human Hair by Ultrasonic Acid Digestion and Atomic Absorption Spectrophotometry

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ABSTRACT

The determination of heavy metals in the biological samples of human beings is important for clinical screening procedure and environmental monitoring. In this study, an ultrasonic acid digestion (UAD) and cloud point extraction (CPE) were developed as a sample preparation and preconcentration for determination of Cr, Ni and Pb in human hair samples. Various parameters that influence an ultrasonic acid digestion, such as acid mixture, presonication time, sonication time and temperature of the ultrasonic bath have been investigated. The suitable conditions for UAD were as follow : an acid mixture of 2 ml concentrated HNO_3 and 1 ml H_2O_2 , 10 min presonication time, 30 min sonication time and 60°C of temperature of the ultrasonic bath. After digestion with UAD, the sample solutions were preconcentrated by cloud point extraction. Chromium, nickel and lead were complexed with ammonium-pyrrolidine dithiocarbamate (APDC) and Triton X-114 was added as non-ionic surfactant. These complexes were extracted into the surfactant-rich phase, at above its cloud point temperature. The surfactant-rich phase was diluted with methanol prior to its analysis by flame atomic absorption spectrophotometry. The chemical variables that affect the cloud point extraction, such as pH of solution, complexing agent concentration, Triton X-114 concentration and complexing time were optimized. Under optimal conditions, the detection limits for Cr, Ni and Pb were 0.077, 0.161 and 0.249 mg ml^{-1} , respectively.

Keywords: human hair, heavy metal, ultrasonic acid digestion, cloud point extraction, atomic absorption spectrophotometer

1. INTRODUCTION

Some heavy metals are essential to maintain the metabolism in human body. However, at high concentration, they can lead to poisoning. The toxic heavy metals such as nickel, cadmium, lead and chromium have negative effect to human health [1]. The human

biological samples which used in analysis such as blood, urine, nail and hair are generally used for investigation of heavy metals. For several years, human hair has been used to assess human exposure to heavy metals, drug abuse, health status and many biological

parameters because toxic heavy metals can be accumulated within hair for a long time. Moreover, hair sample can be collected more quickly and easily than specimens of blood, urine or any other tissue and can be stored and transported to the laboratory for analysis easily. Furthermore, the concentrations of metals in hair sample are high compared with those in body tissues or fluids. This characteristic makes hair an attractive biomonitoring substrate [1-5].

As human hair is a solid sample, a sample pretreatment is usually needed for the analytical determination. Methods based on acid or alkaline digestion are the most widely used for all kinds of solid biological samples [6]. Samples were digested by adding concentrated nitric and perchloric acids in a beaker heated on a hot plate, after which hydrogen peroxide and nitric acids were added [7]. Samples were digested using an oxidant mixture containing nitric and hydrochloric acids and hydrogen peroxide with ultrasound irradiation [8]. Acid digestion has been the pretreatment of choice for the determination of metal in hair samples [1-3, 6-8].

For biological sample digestion techniques, microwave acid digestion has been widely used for total metal determination. However, microwave digestion system gives some drawbacks such as expensive microwave oven, short lifetime of digestion vessel, long time required for cooling digestion vessel and cause of explosion because of high temperature and pressure [9-10]. Therefore, ultrasonic acid digestion (UAD) can be considered as an alternative for solid sample digestion because this technique provides intense and high frequency ultrasound energy to sample through liquid. In addition, samples are mixed effectively and then efficient chemical and physical reactions are provided.

Moreover, there are many advantages of this technique which are short digestion times without applying high temperature and pressure, simple to use, low cost and not special vessel requirement [3, 9-12].

The determination of very low concentration of trace elements usually requires separation and preconcentration steps. The effective separation and preconcentration can solve this problem and provide a higher confidence level. Nowadays, the cloud point extraction (CPE) has been widely used in analytical chemistry. CPE is an attractive technique that reduces the consumption of solvent, disposal costs and extraction time. This research focuses on the development of ultrasonic acid digestion technique with preconcentration by cloud point extraction for determination of toxic metals by atomic absorption spectrophotometry in human hair samples [13-16].

2. MATERIALS AND METHODS

2.1 Apparatus and Reagents

An Analyst 800 atomic absorption spectrometer from Perkin Elmer (Perkin Elmer, Norwalk, USA) was used for Ni, Cd and Pb measurements. A KUDOS-SK250H ultrasonic cleaner (ultrasonic power 250W; frequency 53 kHz) was used for sample preparation. Stock solutions of 1% v/v Triton X-114 (Fluka, Buchs, Switzerland) and $6 \times 10^{-3} \text{ mol l}^{-1}$ Ammonium-pyrrolidine dithiocarbamate (APDC) (Carlo Erba, Italy) were prepared. Acetic acid/sodium acetate buffer solution (pH 4) was prepared using glacial acetic acid (J.T. Baker, Mallinckrodt-Baker, Xalostoc, Mexico) and sodium acetate (Synth, Diadema, SP, Brazil). Stock standard solutions of Ni, Cr and Pb at concentration of $1,000 \text{ mg ml}^{-1}$ were obtained from Fluka, Buchs, Switzerland. Working standard solutions were obtained by appropriate dilution of the stock standard solution. All other reagents were

of analytical-reagent grade (E. Merck, Darmstadt, Germany) and all solutions were prepared with deionized water.

Table 1. Measurement conditions for FAAS.

Parameters	Cr	Pb	Ni
Wavelength (nm)	357.9	283.3	232.0
Slit width (nm)	0.7	1.0	0.2
Lamp current (mA)	4	10	20
Flow rate of air/acetylene(l/min)	8/1.8	8/1.8	8/1.8

2.2 Preparation of Human Hair Samples

Prior to analysis, all hair samples were cut into 1-2 cm with stainless steel scissor and washed according to the procedure described by the International Atomic Energy Agency (IAEA). Human hair samples were first washed with acetone, and then washed three times with DI water and were again washed with acetone one more time. Finally, samples were dried in an oven at 110°C for 16 hrs and kept in a desiccator.

2.3 Ultrasonic Acid Digestion Method

The 200 mg of human hair sample was weighed and put into test tube before adding mixed standard solution of Ni, Cr and Pb for the final concentrations of 3, 2 and 10 mg ml⁻¹, respectively. Then, the concentrated HNO₃ and 30 % H₂O₂ were added. The solution was kept at room temperature for different time intervals (0-60 min), marked as presonication time (PST). After each time intervals of the PST, test tubes were placed inside the ultrasonic bath and were sonicated (ultrasound energy remaining at 53 kHz) for different time interval, termed as sonication time (ST), 5-60 min were applied to samples. The temperature range of ultrasonic bath was 30-80°C. After sonication for different time intervals and temperature, the final

volume was made up to 10 ml in volumetric flasks with deionized water and stored in polyethylene tubes at 4°C for analyses. Blanks were also treated in the same way without samples for each experiment.

2.4 Cloud Point Extraction Procedure

A typical cloud point experiment required the following steps: an aliquot of 5 ml of a sample solution containing analyte ions (3 mg ml⁻¹, 2 mg ml⁻¹ and 10 mg ml⁻¹ for determination of Ni, Cr and Pb, respectively), APDC 2x10⁻⁴ mol l⁻¹, Triton X-114 0.01% (v/v) and acetic acid/sodium acetate buffer pH 4 were subjected to CPE. Then, the volume was made up to 10.00 ml with deionized water. The mixtures were heated at 40°C for 30 min. Separation of the two phases was achieved by centrifuging at 3400 rpm for 10 min. On cooling step in an ice-acetone mixture for 10 min, the surfactant rich phase became viscous and the supernatant aqueous phase was separated. To decrease the viscosity of surfactant-rich phase and facilitate both sample handling and introduction to FAAS, the methanol containing 0.1 mol l⁻¹ HNO₃ was added to make the final volume of 3.5 ml. The solution was introduced by conventional aspiration into a flame atomic absorption spectrometer.

2.5 Analytical Characteristics of the Method

2.5.1 Precision

The precision of the FAAS instrument was studied by analyzing the 3, 2 and 10 mg ml⁻¹ standard solution of Ni, Cr and Pb, respectively, for ten times. The relative standard deviation was calculated.

2.5.2 Detection limit

The detection limits of the method for determination of Ni, Cr and Pb were studied after sample measurement by analyzing

blank for ten times.

3. RESULTS AND DISCUSSION

3.1 Optimization of Ultrasonic Acid Digestion

Variables influencing the ultrasonic acid digestion were optimized within the intervals as shown in Table 2.

Table 2. Operating conditions for ultrasonic acid digestion of Cr, Ni and Pb from human hair samples.

Parameters	Optimal conditions
Solvent systems	
HNO ₃ : H ₂ O ₂ (ml)	
Presonication time(min)	2:1
Sonication time(min)	10
Temperature(°C)	30

3.1.1 Effect of solvent systems

The influence of solvent systems of concentrated HNO₃ and mixture of HNO₃ and H₂O₂ were studied by fixing the other variables at their optimal values. Normally, the digestion is incomplete with only single strong acid. The use of a strong oxidizing acid such as H₂O₂ is necessary. As a result of heating, H₂O₂ dissociates to hydroxyl radicals (OH•) which attack to protein, carbohydrate and poly unsaturated fatty acid in biological sample, hence, it improved the efficiency of the extraction of metals from sample. As shown in Figure 1, the clear solutions were produced after using the H₂O₂ and HNO₃ combined solution and the volume of 2 ml HNO₃ and 1 ml H₂O₂ was chosen for this study.

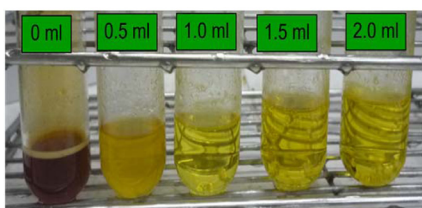


Figure 1. The sample solution with various volume of H₂O₂.

3.1.2 Effect of presonication time

After the treatment with acid-oxidant mixtures human hair samples were kept at room temperature for different time intervals 2-20 min before being subjected to the ultrasonic bath, denoted as presonication (PST). Optimum effects of PST were observed at 10 min. Longer PST has no effect on the recoveries of heavy metals under study.

3.1.3 Effect of sonication time

Before sonication (t=0), the digestion process of human hair solution did not complete. UAD efficiency increased with increasing sonication time. The Figure 2 shows the effect of the sonication time on the analytical signal of the Ni, Cr and Pb. It was observed that optimum signal of Ni, Cr and Pb required 30 min. This fact offers an important practical advantage because the time for the acid digestion can be shortened, and our results are consistent with other study. This optimized condition was applied for further study.

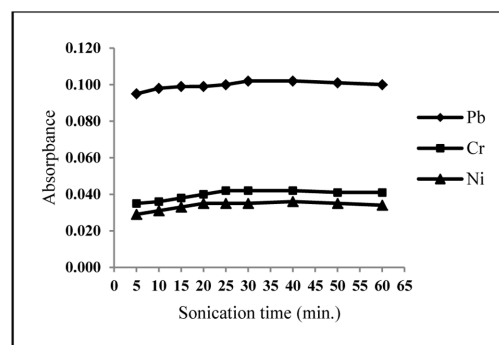


Figure 2. Effect of sonication time on the analytical signal of 3 mg ml⁻¹ Ni, 2 mg ml⁻¹ Cr and 10 mg ml⁻¹ Pb.

3.1.4 Effect of temperature of the ultrasonic bath

The temperature of ultrasonic bath can increase the oxidative power of the acid solution. By sonification, a high kinetic energy of the solid human hair particles and acid solution is reached and the contact between both phases is better. The signals of metals in human hair samples were carried out at different temperatures of the ultrasonic bath (varying between 30-80°C) as shown in Figure 3. It was found that the optimum of temperature of ultrasonic bath was 60°C.

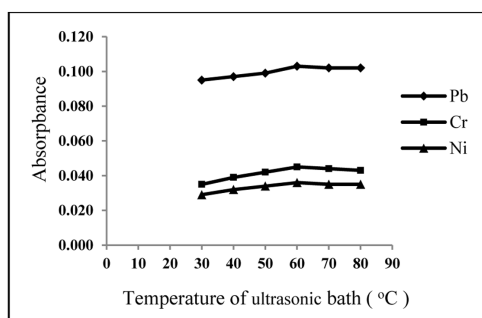


Figure 3. Effect of ultrasonic bath temperature on the analytical signal of 3 mg ml⁻¹ Ni, 2 mg ml⁻¹ Cr and 10 mg ml⁻¹ Pb.

3.2 Optimization of Cloud Point Extraction

Cloud point extraction of metal ions is known to depend on several factors such as type and amount of reagent and surfactant, pH of solution, equilibration temperature and time. We have investigated the CPE process in order to obtain optimum conditions.

3.2.1 Effect of pH

The formation of metal complexes and its chemical stability are the two important influence factors for the CPE, and the pH plays a unique role on metal chelate formation and subsequent extraction. The CPE of nickel, chromium and lead ions was

performed in solutions of pH ranging from 2 to 8. Figure 4 shows the effect of pH on the absorbance and sensitivity of method. It is found that in the pH of 4.0 maximum sensitivity which is an indication of maximum extraction efficiency could be obtained. At lower pH, the ligand is protonated and its ionic characteristics increase and lead to decrease in its solubilization in the hydrophobic micelles. At higher pH, the ligand is deprotonated and it behaves like a hydrophilic molecule and easily gets solubilized in the micelles. In addition, at pH > 4 the precipitation of M(OH)₂ or M(OH)⁺ ions are in the form of hydroxides. Hence the optimum pH value of 4 was chosen for the study.

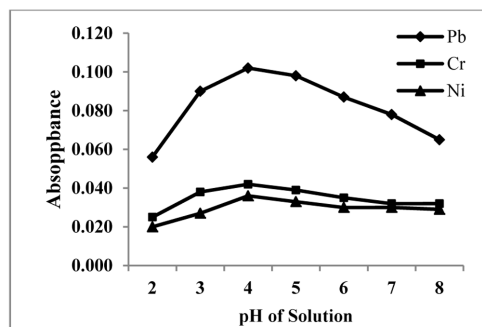


Figure 4. Effect of pH on the cloud point extraction of 3 mg ml⁻¹ Ni, 2 mg ml⁻¹ Cr and 10 mg ml⁻¹ Pb.

3.2.2 Effect of APDC concentration

The effect of the concentration of APDC complexing agent on the CPE efficiency for Ni, Cr and Pb was investigated. The CPE efficiency for metals increased as the concentration of APDC increased from 0.3×10^{-4} to 6×10^{-4} mol l⁻¹ and then kept constant with further increasing in the APDC concentration up to 9×10^{-4} mol l⁻¹ as shown in Figure 5. Therefore, an APDC concentration of 6×10^{-4} mol l⁻¹ was employed for subsequent experiments.

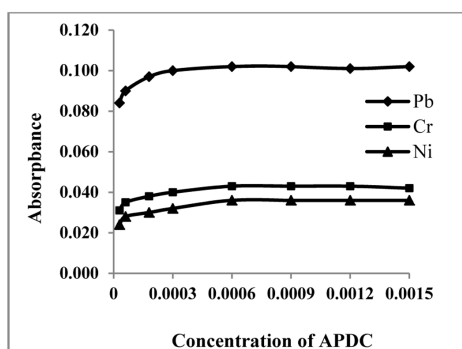


Figure 5. Effect of APDC concentration on the cloud point extraction of 3 mg ml⁻¹ Ni, 2 mg ml⁻¹ Cr and 10 mg ml⁻¹ Pb.

3.2.3 Effect of Triton X-114 concentration

The non-ionic surfactant Triton X-114 was chosen because of its commercial availability in a high purified homogeneous form, low toxicological properties and cost. Also, the high density of the surfactant rich phase facilitates phase separation by centrifugation. The preconcentration efficiency was evaluated using Triton X-114 concentrations ranging from 0.01% to 0.30 % (v/v) as shown in Figure 6. The highest signals of Ni, Cr and Pb were obtained with 0.10% (v/v) Triton X-114 and the signals then decreased at a higher Triton X-114 concentration (0.15 % (v/v)). This result might be related to the presence of a high amount of surfactant, which cause the viscosity of the surfactant-rich phase to increase and lead to poor sensitivity. Therefore, the concentration of 0.1% (v/v) Triton X-114 was selected for the rest of this work.

3.2.4 Effects of equilibration temperature and time

The effects of equilibration temperature and time on the analytical signal were studied in the range of 30-50°C and 10-60 min, respectively. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature, which

compromise completion of the reaction and efficient separation of phases. Therefore a temperature of 40°C and an equilibration time of 30 min which gave the good results were chosen for further experiments. More over higher temperatures lead to the decomposition of complexes, and at lower temperatures the separation of the two phases is not complete.

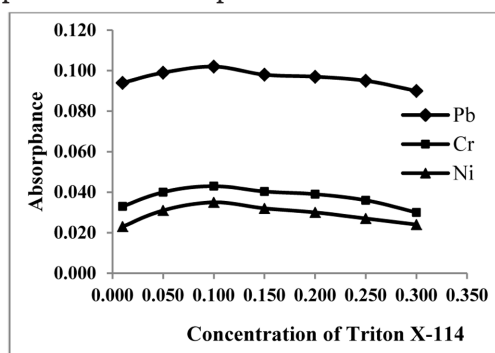


Figure 6. Effect of Triton X-114 concentration on the cloud point extraction of 3 mg ml⁻¹ Ni, 2 mg ml⁻¹ Cr and 10 mg ml⁻¹ Pb.

3.3 Analytical Characteristics of the Method

The precision of the FAAS instrument for Ni, Cr and Pb were found to be 1.59%, 1.27% and 0.81%, respectively. These indicated that the FAAS instrument provided very good repeatability (less than 5 %RSD) for Ni, Cr and Pb under the optimum conditions. The linear range of calibration curves were in the range of 0.50-4.00, 0.50-4.00 and 1.00-20.00 mg ml⁻¹ for Ni, Cr and Pb, respectively.

The detection limits of the proposed method for determination of Ni, Cr and Pb were investigated by analyzing 1% HNO₃ solution for ten times. The detection limits for Cr, Ni and Pb were 0.077, 0.161 and 0.249 mg ml⁻¹, respectively.

3.4 Determination of Cr, Ni and Pb in Human Hair Samples

The proposed method was applied to analyze the human hair samples. For this

purpose, appropriate amounts of Cr, Ni and Pb standard solutions were added into hair sample solutions before digestion. The results in Table 3 show that the ultrasonic acid digestion and cloud point extraction methodology can be applied to the determination of Cr, Ni and Pb in human hair samples by FAAS.

Table 3. The concentration of Cr, Ni and Pb in human hair sample.

Analyte	Sample	Spiked	Found	% Recovery
Cr (mg g ⁻¹)	1	-	3.97±3.44	
		125	126.98±9.09	98.41
		250	242.06±3.44	95.24
	2	-	5.95±0.00	
		125	121.03±3.44	92.06
		250	246.03±6.87	96.03
Ni (mg g ⁻¹)	1	-	19.23±9.62	
		250	266.03±5.55	98.72
		400	394.23±9.62	93.75
	2	-	35.26±5.55	
		250	278.85±0.00	97.44
		400	400.64±5.55	91.35
Pb (mg g ⁻¹)	1	-	4.63±8.02	
		1250	1259.26±8.02	100.37
		2500	2458.33±0.00	98.15
	2	-	18.52±8.02	
		1250	1277.78±0.00	100.74
		2500	2509.26±8.02	99.63

4. CONCLUSIONS

In this study, a safe and rapid digestion method has been established for the reliable extraction of metals in human hair samples. The efficiency of ultrasonic acid digestion has demonstrated a good performance related to solid sample preparation. Several benefits offered by UAD are shortened sample preparation times, simplicity and enhanced safety compared to other more traditional methods. The low costs of the ultrasonic

baths, the UAD can be considered as viable procedures for many laboratories. We have proposed the use of cloud point extraction as an alternative method for the preconcentration of metals as a prior step to their determination at micrograms per milliliter levels in human hair samples by FAAS which is available in most laboratories. The method offers a simple, rapid, inexpensive and nonpolluting alternative to other preconcentration techniques. The presented method can be applied to the determination of metals in human hair samples by FAAS.

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