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On-line solid-phase separation/preconcentration for the determination of copper in urine by flame atomic absorption spectrometry

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ABSTRACT

A new on-line separation/preconcentration system was developed for the determination of Cu(II) ions by flame atomic absorption spectrometry in urine samples. A newly synthesized chelating resin, by anchoring eriochrome blue black R reagent to Amberlite XAD-16 resin, was used as a packing material for the selective separation/preconcentration of Cu(II) ions. The influence of the parameters on the determination of Cu(II) ions such as pH of sample solution, amount of the resin, eluent type, interfering ions and flow variables was studied. The detection limit of the method was 1.0 μg L^{-1} while precision was 2.3% ($n = 15$) at 50 μg L^{-1} Cu(II) level. The adsorption capacity of the resin was 217 μg g^{-1} Cu(II). The accuracy of the method was proven using TMDA-64 standard lake water and synthetic urine sample. The developed method has been applied successfully to the determination of copper in urine with satisfactory results.

1. Introduction

The analysis of biological fluids is one of the most appropriate forms of evaluating environmental exposure to pollutants such as toxic metals. Metals are important constituents widely used in different industrial processes, and can be present in biological fluids, namely urine, as a consequence of occupational exposure. Urine is easily sampled and is an important clinical screening material to assess the possible influence of workplace exposure on workers [1].

Copper is important in the function of some enzymes. It is found naturally in extremely small amounts in some foods, milk for example. But it is frequently added to foods in small amounts. The USDA's "Recommended Daily Allowance" ("RDA") is just 2 mg of Cu [2]. Copper is an essential trace element for the catalytic activity of many enzymes involved in biological processes. In general, copper at nearly 40 μg L^{-1} level is required for normal metabolism of many living organisms. Copper is considered to be toxic at higher levels and severe oral intoxication will affect mainly the blood and kidneys and also depressive pathologies of the central nervous system [3–5].

Under normal conditions the urinary excretion of copper is very low, usually less than 40 μg day^{-1}. However, this value may increase in several pathologies related to abnormalities in copper metabolism. The most important is Wilson's disease, or hepatolenticular degeneration, that results in excessive accumulation of copper in the liver, brain, cornea and kidneys. There is also an increased urinary output of copper, up to 500–1000 μg day^{-1} [6]. Hence, the determination of copper in urine is of particular interest in clinical chemistry for purposes of diagnosis, for monitoring Wilson's patients under chelation therapy, for detection of environmental or occupational exposure, and for nutritional studies. However, this evaluation requires an accurate, selective and sensitive method for the determination of copper [3].

Determination of copper in urine samples is usually performed by atomic spectrometric techniques, such as flame atomic absorption spectrometry (FAAS) [7,8], graphite furnace-atomic absorption spectrometry (GF-AAS) [5,9–11] and inductively coupled plasma-mass spectrometry (ICP-MS) [12,13]. There are some colorimetric methods for the determination of copper in urine, but they are time-consuming and usually require previous ashing and/or chelation followed by extraction [14].

For the determination of copper with on-line solid phase extraction coupled to FAAS, the most efficient chelating agents are diethyldithiocarbamates (DDC) [15–17], ammonium pyrrolidinedithiocarbamate (APDC) [18–21], and diethyldithiophosphate (DDPA) [22].

On-line separation/preconcentration system with a minicolumn of appropriate sorbent has been utilized to enhance sensitivity and selectivity in analytical determination of trace metals [23]. These methods provide an opportunity to avoid contamination and large reagent consumption by working with closed systems. In addition, on-line systems are excellent tools for solution management, allowing the easy implementation of different steps required for selectivity and/or sensitivity enhancement. On-line methods offer higher sample throughput and better precision and accuracy compared to off-line methods. In most on-line FAAS methods, the carrier and reagent solutions are continuously fed to the flow system, mixed on-line and passed through the minicolumn prior to detection [24,25].

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In the present paper, we have evaluated a new on-line system with a home-made controller having five independent channels to adjust the control parameters of the proposed system. For this purpose, Amberlite XAD-16/eriochrome blue black R (AXAD-16/EBBR) chelating resin was used for the on-line separation/preconcentration of Cu(II) ions. To the best of our knowledge the application of AXAD-16/EBBR as a sorbent material in on-line systems was demonstrated for the first time. In the proposed on-line procedure, all the factors effective on the adsorption and elution cycles of the Cu(II) ions were examined. The chelating resin is highly selective for the separation/preconcentration of Cu(II) ions from other interfering ions. The automation of the procedure causes reduction in the risk of operational errors, and increases the speed of analysis and the precision of the method.

2. Materials and methods

2.1. Instrument

A PerkinElmer model AAnalyst 800 flame atomic absorption spectrometer (Norwalk, CT, USA) equipped with a deuterium background correction system and an air–acetylene burner were used for the determination of copper. The wavelength used for copper was 324.8 nm. Spectral bandwidth of 0.7 nm, acetylene flow rate of 1.8 L min^{-1}, and nebulizer flow rate of 10.0 mL min^{-1} were the conventional working parameters. During the signal measurements, the integration time was 0.1 s.

The flow system comprises a single channel peristaltic pump with variable speed (Watson-Marlow Inc., Wilmington, MA, USA), a multichannel peristaltic pump (Ismatec SA, Glattbrugg, Switzerland) furnished with silicone tubings to deliver all solutions, and two three-way valves (Cole-Parmer Co., IL, USA) to select solution ways. Peristaltic pumps (PP) and valves (V) were controlled by a five-channel controller and each channel can be set for 36 different timing periods with thumble switches. This unit was constructed in our laboratory (at low cost) in order to automatically operate the on-line flow injection system. The flow system was constructed using fittings, unions and tees made of plastic and high density polyethylene (HDPE) materials. A home-made glass minicolumn (0.3 cm i.d. and 3.5 cm length) packed with the AXAD-16/EBBR chelating resin was used for the on-line separation and preconcentration of the copper(II) ions. A Consort model C533 pH meter (Consort nv., Turnhout, Belgium) combined with a glass-electrode for pH measurements and a magnetic stirrer model HS31 (Chiltern, Auckland, New Zealand) were used. For the digestion of urine samples, a CEM model Mars XP-1500 microwave oven was used. Functional groups of the synthesized chelating resin were identified with a Shimadzu model FT-IR 8400 spectrophotometer using the attenuated total reflectance (ATR) technique.

2.2. Reagents and standard solutions

All the chemicals were of analytical reagent grade and provided by Merck (Darmstadt, Germany). Deionized distilled water was used for the preparation of the solutions. 1000 μg mL^{-1} Cu(II) solution was prepared by dissolving 1.0 g (to the nearest 0.1 mg) of Cu powder with 2% HNO_3 in a 1-L volumetric flask. From these solutions, diluted working solutions were prepared on a daily basis just before use. 2 mol L^{-1} HNO_3 solution was used as eluent throughout the experiments.

Amberlite XAD-16 resin (Acros Organics, NJ, USA) is a polystyrene divinylbenzene copolymer. In order to remove organic and inorganic contaminants, the resin was washed with distilled water, 1 mol L^{-1} HNO_3 in acetone and distilled water, respectively, and then with water until a neutral solution was obtained. It was dried at 110 °C in an oven and prepared for synthesizing the chelating resin. The EBBR reagent (Merck, Darmstadt, Germany) was used as purchased. The glassware used was cleaned by soaking overnight in dilute HNO_3 (1:5, v/v), and then rinsed with distilled water several times.

The effect of pH of the aqueous phase on the absorbance was examined by varying pH using proper buffer solutions; HCl/KCl for pH 1–2, acetic acid/sodium acetate for pH 3–6, ammonium acetate for pH 7, ammonia/ammonium chloride for pH 8–10, and sodium hydroxide for pH 11–12.

2.3. Synthesis of Amberlite XAD-16-EBBR chelating resin

To prepare the AXAD-16/EBBR chelating resin, the procedure given in the literature was performed [26]. 5 g of Amberlite XAD-16 resin dried at 110 °C was slowly put into a 100-mL of beaker containing a mixture of 10 mL of concentrated HNO_3 and 25 mL of concentrated H_2SO_4 within 30 min by stirring (in a hood). The reaction mixture was held at 60 °C on a water bath by stirring. After cooling, the mixture was poured into a beaker containing ice–water and filtered. The nitrated resin was repeatedly washed with water until free from acid. It was added to a reducing mixture of 40 g of $SnCl_2$, 45 mL of concentrated HCl and 50 mL of ethyl alcohol, and heated thereafter at 90 °C for 12 h under a reflux system. After filtering off, the aminated resin was washed with some water, 50 mL of 2 mol L^{-1} NaOH, and 50 mL of 2 mol L^{-1} HCl, respectively, and then it was washed again with water to remove excess HCl. After the aminated resin was taken into an ice–water mixture, 100 mL of 1 mol L^{-1} HCl and then 75 mL of 1 mol L^{-1} $NaNO_2$ were slowly added to it at 0–5 °C. The diazotized resin was quickly filtered, washed with ice–water and added to the solution of EBBR (5 g of EBBR dissolved in 100 mL of 10% NaOH, w/v) at 0–5 °C for 24 h. The resulting resin beads with brown color were filtered, washed with water, dried in air, and then maintained in a desiccator until usage.

The infrared spectrum of the chelating resin was compared with that of the Amberlite XAD-16 resin. Additional peaks, which not appeared in the spectrum of the Amberlite XAD-16 resin, but appeared at around 3350 (broad band), 1715–1615, and around 1500 cm^{-1} in the spectrum of the AXAD-16/EBBR chelating resin. This confirms that the EBBR ligand was anchored to the polymer support, because the original ligand has characteristic vibrations originating from – OH, aromatic – C═C –, and – N═N – vibrations at these wavenumbers, respectively. The IR spectra and the proposed structure of the chelating resin are given in Fig. 1 [27].

2.4. Preparation of the minicolumn

The minicolumn was prepared by introducing 75 mg of the chelating resin, as a slurry of the polymer beads using a syringe. The ends of the minicolumn were fitted with glass wool to retain the packing material. Before use, ethanol, acetone, 5% (w/v) nitric acid solution and deionized water were passed through the minicolumn at a flow rate of 3.0 mL min^{-1} in order to clean it. The resin bed was approximately 1 cm in length. The packed minicolumn was washed with buffer solutions to condition the AXAD-16/EBBR chelating resin. After each cycle,

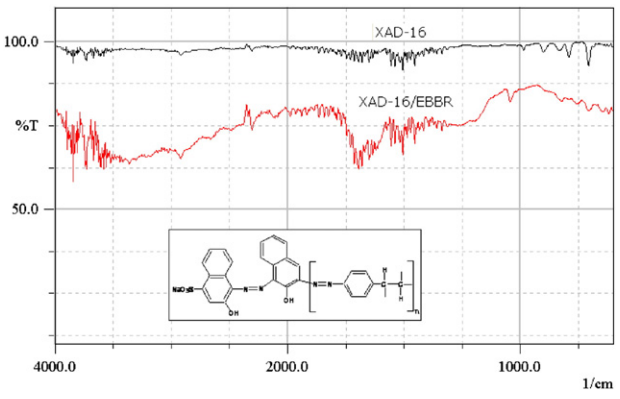

Fig. 1. The IR spectra and the proposed structure of the chelating resin.