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Trace determination of cobalt in biological fluids based on preconcentration with a new competitive ligand using dispersive liquid-liquid microextraction combined with slotted quartz tube–flame atomic absorption spectrophotometry

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ABSTRACT

A new competitive ligand has been synthesized for the preconcentration to obtain lower detection limits by using dispersive liquid-liquid microextraction combined with slotted quartz tube-flame atomic absorption spectrophotometry (DLLME-SQT-FAAS). The proposed method is simple, eco-friendly and has high sensitivity. The preconcentration procedure was optimized on the basis of various parameters affecting the complex formation and extraction efficiency such as pH and volume of buffer solution, volume of ligand solution, mixing period, volume and type of extraction solvent, volume and type of dispersive solvent, and salt effect. Instrumental parameters were also optimized to get higher sensitivity. Under the optimum conditions, the calibration graph was linear in the range of 10–250 ng $mL^{-1}$and the resulted limits of detection and quantification (LOD and LOQ) for combined method were 4.7 and 15.7 ng mL^{-1}, respectively. The detection power was improved 48-fold using DLLME-SQT-FAAS method compared to conventional FAAS. The precision of the method was found to be high with a relative standard deviation of 2.5%. The accuracy of method was evaluated by recovery experiments using matrix matching study on spiked urine and blood samples. The recoveries for urine and blood samples ranged from 99.8 to 108.9% and 102.5 to 110.0%, respectively.

1. Introduction

Cobalt is an essential element for living organisms as a component of vitamin B_{12} which plays an important role for the production of red blood cells and the prevention of pernicious anemia [1]. Insufficient levels of cobalt causes several diseases such as anemia, metabolic disorders, retarded growth and degeneration of nerve cells [2,3]. On the other hand, when it is taken in excessive amounts, this element has been reported as toxic causing goiter, vomiting, diarrhea, increased blood pressure, slowed respiration, giddiness, tinnitus, deafness due to nerve damage and cardiomyopathy [2]. To understand and monitor such toxic effects, there is an increasing need for quantitative information regarding dose and biological levels of cobalt.

In this regard, several quantification methods have been reported including various instrumental techniques such as electroanalytical techniques [4,5], flame atomic absorption spectrometry (FAAS) [6,7], electrothermal atomic absorption spectrometry (ET-AAS) [8,9], inductively coupled plasma optical emission spectrometry (ICP-OES) [10,11] and inductively coupled plasma mass spectrometry (ICP-MS) [12–14] for variety of matrices. Among these techniques, ICP-OES and ICP-MS are the most common for the determination of trace and ultra-trace levels of metals. However, these sophisticated instruments are known to be time consuming, have limitations for complex matrices and they need high investment and expensive operation costs [15]. On the other hand, FAAS is a good choice for trace metal determinations regarding to its simplicity, accessibility and cost-effective analysis [16]. However, the low sensitivity of this method cannot completely compensate other sophisticated systems. In order to improve the sensitivity, a simple attachment called as slotted quartz tube (SQT) has been developed resulting 2–5 fold enhancement in sensitivity which is attributed to the increased residence time of the analyte atoms in the light path [17,18]. Although significant enhancement have been provided with the development of instrumental techniques, they may not provide enough sensitivity in many cases, especially concerning complex

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matrices such as environmental, biological and clinical samples [19]. Therefore, a combined method involving a preconcentration procedure which avoids matrix effects is promising for sensitive, reliable and accurate analytical method(s) [20–22].

Up to now, a wide variety of extraction and preconcentration methods have been used for the determination of many inorganic and organic analytes [23–28]. For cobalt, liquid-liquid extraction (LLE) [29], cloud point extraction (CPE) [30], solid phase extraction (SPE) [31,32] and dispersive liquid-liquid microextraction (DLLME) [33–35] have been applied in literature. Among the extraction/preconcentration methods, DLLME is one of the most effective one regarding to its high recovery, high enrichment factors, rapidity, low cost and low sample volume [36]. DLLME employs a ternary component solvent system comprising the infection of the proper mixture of extraction and dispersive solvent into aqueous sample with the aid of a syringe [36]. Hereby, the droplets are dispersed throughout the aqueous sample resulting the formation of cloudy solution. This rapid and homogenous dispersion allows the extraction of analytes to the interior of droplets. After the centrifugation of the cloudy solution, the analyte(s) is collected in the sediment phase and then determined by using an appropriate instrumental method [37]. In this regard, several ligand structures have been reported with high extraction abilities for toxic heavy metals. Sensitive determination of Co in biological matrices is very important because it is known that the cobalt is not cumulative in body and the blood and urine are mainly reflect cobalt exposure of the human body [38].

In the presented study, DLLME method was used for extraction/preconcentration of cobalt by using a novel ligand. After extraction procedure, sensitive and selective determination was achieved by using a SQT-FAAS system. All the system parameters were optimized and the developed method was successfully applied to blood and urine samples. To the best of our knowledge, no other previous report has been published for the determination of cobalt by using DLLME-SQT-FAAS method in blood and urine samples.

2. Materials and methods

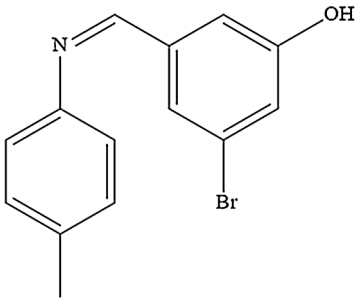
2.1. Instrumentation

All measurements for the determination of cobalt were carried out using an ATI UNICAM 929 AA model flame atomic absorption spectrometer combined with a lab-made SQT equipment with the dimensions of 16 cm tube length, 3.2 cm and 5.5 cm upper and lower slot length (positioned 180° with respect to each other); 1.5 cm and 1.8 cm inner and outer diameters, respectively. SQT was placed on the burner head and aligned in the optical path of the spectrometer. A cobalt hollow cathode lamp was used as the radiation source operating at 240.7 nm with a current of 7.0 mA and a slit width of 0.2 nm. An air/acetylene mixture was used to generate the flame. Background corrections were performed by a deuterium (D_2) lamp. A Milestone microwave digestion system was used for the digestion of blood and urine samples.

Characterization of synthesized ligand were acquired by ^{1}H NMR, ^{13}C NMR, FTIR, and LC–MS (QTOF) systems. IR spectra were obtained with a Perkin Elmer Spectrum 100 FT-IR system. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III-500 MHz NMR spectrometer. High-resolution mass spectra (HRMS) were acquired in the positive ion mode using an Agilent G6530B QTOF mass spectrometer.

2.2. Chemicals and reagents

All the chemicals and reagents used throughout the experiment were of high purity grade. Cobalt standard stock solution of 1000 mg L^{-1} was obtained from High Purity Standards-USA. Ethanol, methanol, 2-propanol, chloroform, 1,2-dichloroethane, dichloromethane, carbon tetrachloride, nitric acid, sodium chloride, potassium chloride, potassium nitrate, potassium iodide, hydrochloric acid (HCl), sodium tetraborate decahydrate and sodium sulfate were all purchased from Merck (Darmstadt, Germany). Ultrapure water from a Milli-Q® Reference Ultrapure Water Purification System with the resistivity of 18.2 M-ohm-cm was used for the preparation of standard and sample solutions.



(*Z*)-3-bromo-5-((*p*-tolylimino)methyl)phenol

Fig. 1. Structure of synthesized ligand.

2.3. Synthesis of complexing agent

Synthesis of ligand was carried out according to the following procedure: 10 mmol of 5-bromosalicylaldehyde was dissolved in 25 mL of ethanol. Then, a 0.01 mg of p-toluenesulfonic acid was added to the solution and resulted mixture was heated to 60 °C. 20 mmol of p-toluidine was added dropwise to the final solution after dissolved in 25 mL of ethanol. The reaction color was turned to dark orange resulting precipitation of the ligand. Product was filtered and dried in oven at 50 °C. The structure of the ligand was given in Fig. 1.

2.4. Procedure

The developed analytical method is based on the complexation of cobalt with a new complexing agent and the extraction of metal complex by using the mixture of isopropyl alcohol as dispersive solvent and 1,2-dichloroethane as extractor solvent. Ligand was prepared in ethanol with the concentration of 0.10% w/v. Buffer solution (pH 9) was prepared by mixing 50 mL of 0.025 M sodium tetraborate decahydrate and 4.6 mL of 0.1 M HCL. Before the extraction process, 1.0 mL of buffer (pH 9) and 1.0 mL of ligand solution was added to 8.0 mL of standard/sample solution. The mixture was vortexed for 15 s to obtain a homogeneous dispersion of ligand throughout the solution. 2.0 mL of 2-propanol and 300 µL of 1,2-dichloroethane were mixed in a separate tube and injected into the cobalt complex solution with a syringe, rapidly. The resulted cloudy mixture of the solution was vortexed for 15 s. As the surface area between the aqueous and extraction phase was very high, cobalt complex easily transferred into the fine droplets of extractor solvent. Finally, the solution was centrifuged for 2.0 min at 6000 rpm and the sediment phase was transferred to a clean tube and dried using a water bath at 100 °C. Before analysis, the sediment was dissolved with 250 µL of concentrated nitric acid.

2.5. Sample preparation

Samples were obtained from volunteer laboratory staff. About 0.50 g of blood sample and 4.0 mL of urine sample were taken into the vessels. Concentrated nitric acid and hydrogen peroxide solutions were mixed (1:1 for urine, 3:1 for blood) and 8.0 mL of acid mixtures were added to samples. The sample heating program of microwave oven used for both samples is given in Table 1. After the digestion, oven was left to cooling and the final solution were adjusted to pH 9.0 with 1.0 M NaOH. Final solution was diluted with deionized water.