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Streptomyces levoris Immobilized on Silica Gel 60 as a Novel Biosorbent for Copper (II) Preconcentration

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Abstract. In the present study dead *Streptomyces levoris* biomass loaded on silica gel 60 was applied as an eco-friendly solid phase extractor for copper (II) preconcentration prior to its determination by flame atomic absorption spectrometry. The influences of different parameters such as pH of the sample solution, amount of solid phase, type and concentration of eluent, flow rate of sample solution, sample volume, and interfering effect of diverse ions on the preconcentration procedure were evaluated. An enrichment factor of 25 was achieved under optimum experimental conditions. The obtained results showed that *Streptomyces levoris* immobilized on silica gel can be considered as a promising new biosorbent for solid phase extraction of trace amounts of copper (II).

Key words: *Streptomyces levoris*, preconcentration, trace metal, atomic absorption spectrometry.

Introduction

Copper has received considerable attention because it is the most widely used chemical element in mechanical engineering, electronics, construction, metallurgy and chemical industry. Moreover, it is a trace element present in all tissues and is required for cellular respiration, peptide amidation, neurotransmitter biosynthesis, pigment formation, and connective tissue strength. Copper is a cofactor for numerous enzymes and plays an important role in the central nervous system development. Low concentrations of copper may result in incomplete development, however excess copper intake leads to serious health problems such as severe mucosal irritation, damages of capillary, hepatic, renal and central nervous system (DESAI & KALER, 2008; KALAVATHY *et al.*, 2005). For these reasons the precise determination of copper at low concentrations in natural water and biological samples is very important.

Various instrumental techniques like spectrophotometry, voltammetry, inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), X-ray fluorescence (XRF), electrothermal atomic absorption spectrometry (ETAAS) and flame atomic absorption spectrometry (FAAS) are used and continuously employed for determination of traces of heavy metal ions and copper, respectively. The ease of operation, high precision, selectivity, and low cost make FAAS one of the most frequently used instrumental technique for determination of trace metals (SVRAKA *et al.*, 2014; NGEONTAE *et al.*, 2009). However, because of the high detection limits of FAAS (or low instrumental sensitivity), as well as the interfering effect of the sample matrix the determination of trace metals in water samples is usually associated with a step of preconcentration of analytes (SOYLAK *et al.*, 2005; BUDIZAK *et al.*, 2003). The most widely used preconcentration techniques include precipitation and co-precipitation (DURAN *et al.*, 2014; FEIST & MIKULA, 2014), liquid-liquid extraction (SARAN *et al.*, 1992; ABKENAR *et al.*, 2010), membrane filtration (SOYLAK *et al.*, 2004; DIVRIKLI *et al.*, 2007), ion exchange (KENEWY *et al.*, 2000), electrochemical methods (BULSKA, 2001), solid phase adsorption (SAÇMACI *et al.*, 2011; CHALAPATHI, 2012), etc.

The solid phase extraction is the most frequently used preconcentration technique and can be easily used with FAAS without laborious procedures. It offers several advantages such as flexibility in solid phase selection, low cost due to the minimal reagent consumption, high preconcentration factor, improved sensitivity, no requirements of toxic solvents, speed, simplicity and the ability for automatisation.

Inactive or dead microbial biomass can serve as a new biosorbent material capable to concentrate and recover heavy metals even when they are in concentrations less than 100 ppm. Different types of microorganisms (fungi, yeast, bacteria) and algae, immobilized on natural and synthetic adsorbents have been used for preconcentration, matrix separation, and speciation analysis of heavy metals in trace levels. These new biosorbents are not only selective, efficient and cheap, but they can be regenerated for multiple use or reutilized and are competitive with artificial resins and sorbents for metal concentration (PEREZ-CORONA *et al.*, 1997; BĂG *et al.*, 1998; BAYTAK & TÜRKER, 2004, 2005a; BAYRAMOGLU *et al.*, 2005; BAYTAK *et al.*, 2006; DOGRU *et al.*, 2007).

The *Streptomyces* genus belongs to Gram positive bacteria (EL-SAYED *et al.*, 2011). Three main components – peptidoglycan, teichoic acids and surface proteins, consist the *Streptomyces* cell wall. All of these polymers could play an important role in metal ions sequestration (CHOJNACKA, 2010; SAURAV & KANNABIRAN, 2011). The use of *Streptomyces* biomass as a sorbent in solid phase extraction is poorly studied. The first study is reported by YILDIZ *et al.* (2013). Their research proposes the use of *Streptomyces albus* immobilized on sepiolite as a biosorbent for preconcentration of Cd, Zn and Ni prior their analysis by flame atomic absorption spectrometry.

The aim of this study was to use *Streptomyces levoris*, loaded on silica gel, as an eco-friendly biosorbent for preconcentration of copper in water samples prior to its determination with FAAS.

Materials and Methods

Reagents and solutions

Deionized water was used to prepare all solutions. All solutions and chemicals used were of analytical reagent grade. The copper (II) working solutions were prepared daily by diluting a corresponding 1000 µg dm^{-3} solution (Merck). The pH values of the sample solutions were adjusted to a range of 2–9 with HCl or NH_3. The HCl and HNO_3 solutions used as eluents were prepared by direct dilution from the concentrated solutions. The laboratory glassware was kept overnight in a 5% (v/v) HNO_3. Afterwards, it was rinsed thoroughly with deionized water and dried.

Instrument

A Perkin-Elmer PinAAcle 900 T atomic absorption spectrometer was used. The apparatus was run in flame mode under the conditions suggested by the manufacturer, i.e.: wavelength, 324.8 nm; bandwidth of the slit, 0.7 nm; air/acetylene flow rates, 10 and 3.3 dm^3 min^{-1}. A pH meter model WTW inoLab pH 720 was used to measure the pH of the solutions.

Microorganism and growth conditions

Levorin producing strain *Streptomyces levoris* was provided by Department of Biochemistry and Microbiology, Plovdiv University "Paisii Hilendarski". Liquid culture medium was used for development of the culture with the following composition in g dm^{-3}: glucose – 2.0; starch – 1.0; $(NH_4)_2SO_4$ – 0.8; KH_2PO_4 – 0.01; KCl – 0.1; $MgSO_4$ – 0.25; $CaCO_3$ – 0.3. For inoculation 2% (v/v) spore inoculum was used with 2.10^9 cm^{-3} concentration of the spores. The cultivation was conducted in 500 cm^3 Erlenmeyer flasks containing 50 cm^3 of the culture medium at pH 7.2, at 28±2°C on a rotary shaker at 220 rpm for 96 h. After 96 h of incubation, at the end of the exponential growth phase, the biomass was separated from the medium by vacuum filtration and heat inactivated at 120°C for 20 min at a pressure of 1 atm (STANCHEV *et al.*, 2010). The biomass was washed several times with deionized water until pH 6 was obtained then was treated with 0.1 mol dm^{-3} HCl solution for 30 min. The mixture was centrifuged at 4000 rpm, the resulting biomass was dried to constant weight at 80°C. The obtained biomass was stored at 4°C until further use.

Immobilization procedure

Streptomyces levoris was immobilized in Silica gel 60 (Merck 35–70 mesh). Before use the surface of the silica gel was activated – 4 g of silica gel and 30 cm^3 HCl (6 mol dm^{-3}), were heated at reflux while stirring for 8 hours, then the silica gel was filtered, washed with distilled water until neutral pH was achieved and dried under vacuum at 70°C for 8 h. Then 1g of the activated silica gel was mixed with 300 mg dry and dead biomass powder. The biomass was immobilized by the procedure proposed by MAHAN & HOLCOMBE (1992). The silica gel and biomass mixture was wetted with 2 cm^{-3} of water and thoroughly mixed. After mixing, the resulting paste was heated in an oven at 105°C for 1 h to dry the mixture. In order to accomplish maximum contact between the *Streptomyces levoris* biomass and the silica gel, and to improve the immobilization efficiency the wetting and drying steps were repeated several times. The resulted immobilized biosorbent was marked as SL.

Column preparation

The column used for the preconcentration was 1 cm in diameter and 10 cm long. A small amount of glass wool was placed at the both ends of the column in order to hold the immobilized biomass. The bed height of the SL biosorbent in the column was approximately 10 mm. The column was connected with a peristaltic pump (Masterflex, Cole-Parmer), which provide a continuous flow of the liquid samples. Before use, 1 mol dm^{-3} HCl solution and deionized water were passed through the column in order to clean and activate it. The column was conditioned to the studied pH by passing aqueous solutions of HCl or NH_3 with the same pH as the sample.

General preconcentration procedure

An aliquot of a sample solution containing 10 µg of Cu (II) was taken and the pH was adjusted to the optimum value. The resulting solution was passed through the column at a flow rate of 3 cm^3 min^{-1}. Then, the retained metal ions were eluted with suitable eluent, determined experimentally at flow rate of 1 cm^3 min^{-1}. The copper concentration was determined by FAAS. The biosorbent was used repeatedly after washing with 1 mol dm^{-3} HCl and deionized water. The analyte recovery was calculated from the ratio of the concentration found by FAAS to that calculated theoretically.

Results and Discussion

To obtain the maximum recoveries and to determine the applicability of the method, different parameters such as pH of sample solution, amount of biosorbent, type and concentration of eluent, sample volume and flow rate of sample solution were optimized. Interfering effects of common coexisting metal ions were also studied.

Effect of pH on recovery of copper (II)

The first variable optimized was the pH of the sample solution, because pH is the major factor influencing the metal biosorption process, the surface charge of the biosorbent and the solution chemistry of the metals. The effect of pH of the solution on the copper (II) recovery was studied at pH ranging from 2.0 to 9.0 and the obtained results are shown in Fig. 1.

Quantitative recoveries (>95%) were obtained at pH 6.0 – 7.0. At pH 6.0 the calculated recovery was 98±2.4%.

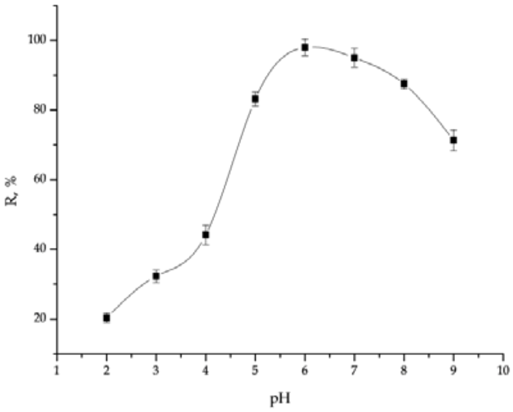

Fig. 1. Effect of pH on copper (II) recovery (sample volume –50 cm^3; amount of copper – 10 µg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl solution; flow rate of sample – 3 cm^3 min^{-1}; flow rate of eluent – 1 cm^3 min^{-1}).

At lower pH, the copper recovery decreased, due to the electrostatic repulsion of the protonated active sites of the biosorbent with the positive charged copper species. The increasing of the pH caused the deprotonation of these groups and formed negatively charged sites for electrostatic attraction of the positively charged metal ions. For higher pH values, the retention decrease and at pH 9.0 the calculated recovery was 71.3±3%. This result could be explained with the competition between the hydroxylated complexes of the metal and active sites of the cell. Similar pH dependences were obtained with *Saccharomyces cerevisiae* immobilized on sepiolite for column preconcentration of Fe (III) and Ni (II) (Bağ *et al.*, 1998), and *Agrobacterium tumefacients* immobilized on Amberlite XAD-4 for iron (III), cobalt (II), manganese (II) and chromium (III) preconcentration (Baytak & Türker, 2005b).

Effect of biosorbent amount

The amount of SL biosorbent is another important parameter that affects the metal recovery. A quantitative retention could not be obtained when the amount of solid phase is less, but an excess amount of sorbent prevents the quantitatively elution of the retained analyte by the small volume of the eluent (Inkaya *et al.*, 2010).

With the increasing of the mass of the SL biosorbent in the range from 100 to 400 mg, the recoveries of the metal ion also increased from 73±3.0 to 98.4±1.8%, respectively (Table 1). Therefore, 300 mg of immobilized biosorbent was used for subsequent experiments.

Table 1. Effect of amount of the immobilized biosorbent on copper (II) recovery (n=3).

Amount of biosorbent, mg	Recovery, %
100	73.0±3.0
200	85.2±2.2
300	98.0±2.4
400	98.4±1.8

Type and concentration of eluent

The effectiveness of the solid phase extraction depends on the type and the concentration of the eluent. The most frequently used eluents are acids and to prevent the biomass degradation it is preferable to be used solutions with lower concentrations (Bağ *et al.*, 2000). Two inorganic acids, HCl and HNO_3, were examined as a suitable eluents. The effect of different volumes and concentrations of HNO_3 and HCl were tested to remove the absorbed copper (II) ions from the bacterial biomass loaded into the column. The obtained results (Table 2) showed that copper ions could be eluted efficiently with 10 cm^3 of 1 mol dm^{-3} HCl and this eluent was chosen in subsequent experiments.

Effect of flow rate

The flow rate of the sample solution through the column is another important parameter, since does not only affectthe recovery of the analyte, but also influences the analysis time. The recovery of the copper (II) ions was studied at flow rates from 2 to 6 cm^3 min^{-1}. The obtained results (Fig. 2) showed that flow rates in range from 1 to 4 cm^3 min^{-1} had no significant effect on the recoveries of the copper; this could indicate that the copper (II) sorption is a rapid process. At flow rate equal and higher than 5 cm^3 min^{-1}, the recovery decrease, because the copper ions cannot be sorbed sufficiently. Optimal flow rate 3 cm^3 min^{-1} was determined. The obtained results are in accordance with other researches (BĂG *et al.*, 1998; BAYTAK & TÜRKER, 2005a, 2005b; BAYTAK *et al.*, 2005).

Table 2. Effect of type and volume of eluent on the recovery of copper (II) (n=3)

Type and concentration of elution solution	Volume, cm^3	Recovery, %
HCl, 0.5 mol dm^{-3}	5	44.3±2.2
	7	52.7±2.2
	10	74.1±1.8
HCl, 1 mol dm^{-3}	5	80.1±1.6
	7	93.6±1.4
	10	98.0±2.4
HNO$_3$, 0.5 mol dm^{-3}	5	38.1±1.4
	7	42.3±2.6
	10	58.0±4.2
HNO$_3$, 1 mol dm^{-3}	5	56.3±2.8
	7	60.5±2.2
	10	76.8±3.2

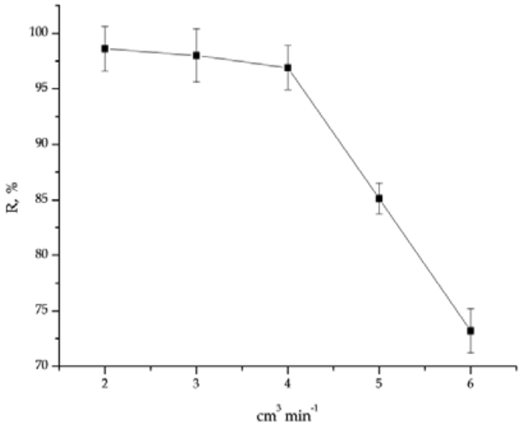

Fig. 2. Effect of sample flow rate on copper (II) recovery (pH 6.0; amount of copper – 10 µg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl solution; flow rate of eluent – 1 cm^3 min^{-1}; sample volume –50 cm^3)

Effect of sample volume

The influence of the sample volume on the recoveries of the copper ions was also examined in order to determine the maximum applicable sample volume and the minimal analyte concentration. Copper (II) ions were preconcentrated, under optimal conditions (pH, eluent type, flow rate, etc.), from the sample solutions that containing 10 µg of copper in 50, 100, 250, 500 and 1000 cm^3, which corresponds to copper concentrations of 0.2, 0.1, 0.04, 0.02 and 0.01 µg cm^{-3}, respectively. The obtained results are shown in Fig. 3.

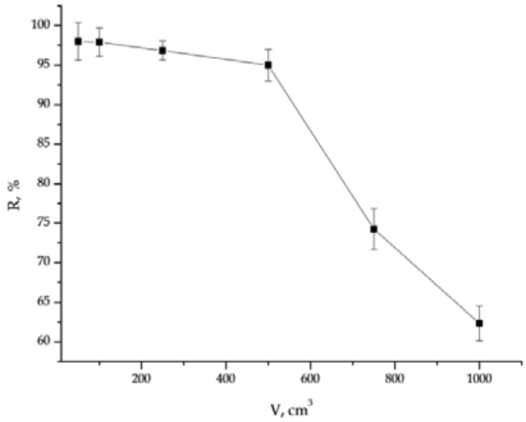

Fig. 3. Effect of sample volume on copper (II) recovery (pH 6.0; amount of copper – 10 µg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl solution; flow rate of sample – 3 cm^3 min^{-1}; flow rate of eluent – 1 cm^3 min^{-1}).

The recovery of copper ions was quantitative (>95%) when the sample volumes were below 250 cm^3. At higher volumes, the recoveries decreased. Since the original sample volume and final volume of solution (after preconcentration) are 250 cm^3 and 10 cm^3, and an enrichment factor of 25 was achieved. These results showed that copper could be determined even in concentration of 0.04 μg cm^{-3} by the proposed method, which could not be determined directly by FAAS with satisfactory precision.

Interference studies

One of the main problems in the atomic absorption spectrometric determination of heavy metal ions is the matrix interference. In water analysis the most common coexisting metal ions are Na^+, Ca^{2+}, Mg^{2+} and often their concentrations exceed 2-5 orders the concentration of the toxic metal. Therefore, the influence of interfering ions on the solid phase extraction of copper (II) ions was studied. Metal ions (Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+}) were added individually to copper solution as their nitrate or chloride salts (NaCl, KCl, $CaCl_2$, $MgCl_2$, $Zn(NO_3)_2$) and the proposed preconcentration method was applied. No interfering effect was found on the recovery of the copper up to 250 mg dm^{-3} of Na^+, 5 mg dm^{-3} of Mg^{2+}, 100 mg dm^{-3} of K^+, 20 mg dm^{-3} of Ca^{2+} and 10 mg dm^{-3} of Zn^{2+} ions. At these concentrations of the coexisting metal ions the copper recoveries were 96±3, 95±2, 96±4, 95±3, 96±3, respectively. These data showed that the proposed preconcentration method could be applied to natural water samples that contain such ions at the tolerable levels listed above.

Column stability

At optimum experimental conditions the stability of the biosorbent loaded column was tested with several cycles of adsorption and desorption, done by passing 100 cm^3 of copper solutions through the column. Testing the column stability showed (Fig. 4) that the sorption capacity after 10 cycles of sorption and desorption does not vary more than 2.0%. Therefore, repeated use of the resin is possible.

Analytical figures of merit

To determine the detection limit (LOD) of the proposed method, 50 cm^3 of a blank solution (distilled water) was passed through the column and the retained metal ions were eluted with 50 cm^3 of 1 mol dm^{-3} HCl solution. This procedure was repeated 10 times.

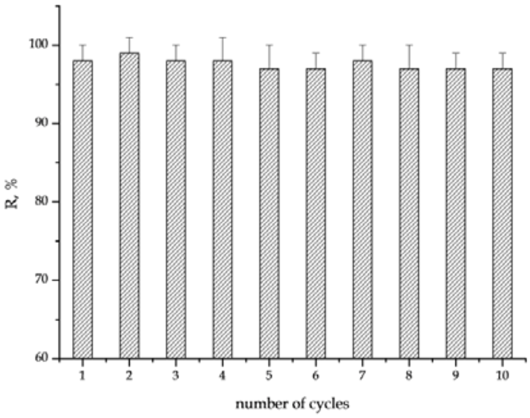

Fig. 4. Stability of SL column for preconcentration of copper (II) ions (pH 6.0; amount of copper – 10 μg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl; flow rate of sample – 3 cm^3 min^{-1}; flow rate of eluent – 1 cm^3 min^{-1}; sample volume – 100 cm^3).

The LOD and the limit of quantification (LOQ) were calculated by using equations (1) and (2) (BĂG *et al.*, 1998; BAYTAK *et al.*, 2005):

$$LOD = [3\sigma / m] / PCF \tag{1}$$

$$LOQ = 3LOD \tag{2}$$

where: σ – standard deviation of the blank signal; m – slope of calibration curve; PCF – preconcentration factor.

The linear range for copper determination, without the preconcentration system was between 0.1 and 5 μg cm^{-3}. After preconcentration of the copper (II) ions a LOD of 1.39 μg dm^{-3} was found. The limit of quantification (LOQ) was calculated as 4.17 μg dm^{-3} by considering 3 times the LOD value. These concentrations of copper cannot be determined directly by FAAS with sufficient accuracy and precision.

For the determination of the repeatability of the method, six successive retention and elution cycles were performed with 100 cm^3 of sample solution containing 10 μg Cu (II) at the optimum conditions. The recovery of Cu (II) is quantitative – 98.0±2%, the calculated relative standard deviation was 2.04%.

Application

The proposed method was applied to preconcentrate and determine the copper (II) ions content in spiked samples of mineral water. Water sample – a commercially available mineral water, was filtered through a Millipore cellulose membrane filter with 0.45 µm pore size. The pH of the filtered water sample was adjusted to 6.0 with 1 mol dm^{-3} HCl solution and the sample was passed through the column. The copper adsorbed on the SL biosorbent was eluted with 1 mol dm^{-3} HCl and the concentration of the analyte was determined by FAAS. The results are shown in Table 3.

Table 3. Determination of copper (II) in mineral water (sample volume 250 cm^3; mean of five determinations at 95% confidence level).

Added, µg dm^{-3}	Found, µg dm^{-3}	Recovery, %
-	7.9± 0.5	-
10	17.7± 0.2	99
20	27.6± 0.4	99

Conclusions

The proposed SPE procedure provides a simple, selective, accurate, precise and eco-friendly method for preconcentration and determination of copper in large volumes of various sample solution. The main advantages of the proposed procedure is the minimal consumption of reagents, low limits of LOD and LOQ, high tolerance to interfering ions quick and easy preparation of the extraction system, and a good preconcentration factor of 25. The operating stability of the system is good and no organic reagents are used. Disadvantages of the proposed preconcentration procedure are the narrow pH range for the quantitative recovery of the copper ions and the time needed to reach the preconcentration factor of 25, is in the order of 120 min.

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