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A voltammetric *Rhodotorula mucilaginosa* modified microbial biosensor for Cu(II) determination

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ABSTRACT

It is the first report about the usage of *Rhodotorula mucilaginosa* as a biomaterial to construct a microbial biosensor based on carbon paste for determination of copper. Cu(II) was preconcentrated electrode surface at open circuit and then detected with electrochemical techniques, including Cyclic Voltammetry (CV) and Differential Pulse Stripping Voltammetry (DPSV). Some parameters such as pH of preconcentration solution, preconcentration time, scan rate and effect of interfering heavy metal ions were carried out for optimum responses. The best defined cathodic peak was obtained at pH 5 with 0.05 M $NaNO_3$ and a scan rate of 100 mV/s. The linear range for the developed microbial biosensor was found in the range of 1.0×10^{-7} and 1.0×10^{-5} M (0.0064 and 0.64 mg/L) at the response time of 15 min ($R^2=0.98$). The easy fabrication, sensitivity, low cost and fast response time showed the advantages of the biosensor to conventional techniques.

1. Introduction

Heavy metal determination in environmental control is usually performed by spectroscopic, voltammetric or chromatographic methods which are able to detect single elements or species at low concentrations, but can hardly be used for in-situ analysis or low cost screening procedures. On the contrary, these tasks can be successfully accomplished by biosensors [1]. Using biosensors for quantitative analysis offers an alternative to traditional toxicity tests and classical analytical methods. Biosensors allow a fast, on-site measurement of metal ion concentration by using low cost compact portable equipment offering the same sensitivity and accuracy as the routine techniques do.

Virtually all organisms require copper as a catalytic cofactor for biological processes such as respiration, iron transport, oxidative stress protection, peptide hormone production, pigmentation, blood clotting and normal cell growth and development. However, copper also participates in redox reactions that generate the hydroxyl radical, which causes catastrophic damage to lipids, proteins and DNA. Consequently determination and environmental control of copper should be taken into consideration [2].

In the development of biosensors some biologically active materials such as enzymes, cells, plant and animal tissue have been mostly used. Because of their high specific activities and analyte sensitivities, enzymes are mostly used in the biosensor construction. But most of enzymes used in the microbial biosensor construction are unstable and costly for routine analysis of the specific substances [3]. On the other hand, whole cell microbial sensors have received recent attention; because enzyme purification is unnecessary, whole cell microbial sensors are simple and inexpensive systems to construct biosensors and enzymes are usually more stable in their natural environment in the cell [4]. In addition to these, microbial biosensors are easy to operate and their short responsive time makes them suitable for online and field monitoring [5,6].

The use of biomasses as modifying agents for the preparation of sensors has attracted much interest. The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Cu, Cr, Ni, U and Zn from aqueous solutions. Most of yeast can adsorb a wide range of metal ions or be strictly specific in respect to only one metal ion [7–9]. In addition, there are some advantages to use yeast in the biosensor construction such as speed of growth on a variety of different carbon sources. Yeast is particularly robust with a wide physicochemical tolerance, e.g. pH, temperature and ionic strength and tough cell [3,7].

This study describes a microbial biosensor for selective, not time consuming Cu(II) determination based on carbon paste electrode modified with a lyophilized yeast cell, *Rhodotorula mucilaginosa*. There are a lot of methods related to electrochemical detection of heavy metals using ion exchange voltammetry, but a few studies taking place in literature about the usage of yeasts for determination of heavy metals at biosensor applications. As far as we know, application of biosensor based on carbon paste electrode modified

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with *R. mucilaginosa* for determination of copper from aqueous solutions has not been reported yet.

R. mucilaginosa was selected because of its strong affinity toward copper. Bioaccumulation capacity of *R. mucilaginosa* for Cu(II) has been studied before. The promising results were found for removal of high concentrations of copper ions from the solutions by metal uptake property of *R. mucilaginosa* cells [10]. The present method is reasonably selective and has short responsive time. It is also simple to prepare and does not require excess preliminary treatment.

2. Materials and methods

2.1. Chemicals

All solutions were prepared with distilled water. Stock solutions of Cu(II), Ni(II), Zn(II) and Pb(II) (1.0×10^{-3} M) were prepared from the corresponding analytical grade metal nitrates (Merck, Darmstadt, Germany). Standard solutions of metal ions were prepared freshly by diluting stock solutions. Electrolyte solution was prepared from the analytical grade $NaNO_3$ (Merck, Darmstadt, Germany). Graphite powder (−300 mesh) and paraffin liquid (Merck, Darmstadt, Germany) were used for preparing the carbon paste electrode (CPE). Yeast Peptone Glucose medium (YPG) was used for cultivation of culture. Other chemicals used for cultivation medium were also prepared from analytical grade. The pH of preconcentration solutions was set using 0.1 M KOH or 0.1 M HNO_3 (Merck, Darmstadt, Germany).

2.2. Microorganism and culture conditions

R. mucilaginosa was used in this study as biomaterial to construct microbial biosensor and obtained from Culture Collection of Biotechnology Research Laboratory, Biology Department, Ankara University, Turkey.

Yeast Peptone Glucose (YPG) medium was used for cultivation. Microorganism was incubated for 5 days at 30 °C on a rotary shaker at 100 rpm (New Brunswick Scientific Innova 4230). After incubation period, the cells of *R. mucilaginosa* were harvested by centrifugation at 5000 rpm for 10 min. The supernatant was discarded and washed distilled water. Centrifugation and washing process was repeated three times and then obtained biomass was lyophilized (Edwards, England).

2.3. Preparation of microbial biosensor

The modified CPE was prepared my making a homogenous paste with various amounts of lyophilized cell, graphite powder and paraffin liquid. The paste mixture firmly packed into the hole of electrode body (0.5 cm^2) and pressed with a steal rod. Then electrode surface was smoothed on a paper.

2.4. Apparatus and experimental method

Electrochemical experiments were performed at room temperature with CH Instruments 660B model Electrochemical Analyzer (CH Instrument USA). The instrument was equipped with conventional three electrode cell containing supporting electrolyte solution, 0.05 M $NaNO_3$, deoxygenated by bubbling pure nitrogen before measurements. Working electrodes were carbon paste electrodes modified with *R. mucilaginosa*. The counter electrode was a platinum wire and the reference electrode an Ag/AgCl (saturated KCl). The pH values were measured with a pH meter (Consort P 500).

Experiments were done using the accumulation, medium change and voltammetry scheme. In the accumulation step the modified CPE was immersed in a stirred copper ion solution for a selected time at open circuit. After this step, biosensor was taken out and surface washed carefully with distilled water. Finally, biosensor was placed in the electrochemical cell filling with 15 ml of 0.05 M $NaNO_3$ and voltammetrically measured. Cyclic voltammetry assays were performed with both modified and unmodified CPEs. A 5 mg of lyophilized biomass (5.0% (w/w)) was used for making paste and potential range of −600 to 800 mV was employed and to back (versus the Ag/AgCl reference electrode). Scan rates of 100 mV/s was used in the analysis. For Differential Pulse Stripping Voltammetry assays, modified CPE was prepared with a 10 mg lyophilized biomass (10% (w/w)) and the other technique parameters were identical with CV assays. The background current was also obtained after the accumulation step in the absence of Cu(II) (blank solution) at open circuit.

2.5. SEM measurements

The surface morphology of the microbial biosensor was examined by Scanning Electron Microscopy and elemental analysis was taken out with Energy Dispersive X-ray Spectra (JEOL JSM-7000F Field Emission SEM-EDAX).

3. Results and discussion

3.1. Cyclic voltammograms of microbial biosensor

Cyclic voltammograms of unmodified and modified CPEs were obtained in 0. 05 M $NaNO_3$ for 1.0×10^{-3} M Cu (II). The cyclic behaviors of the biosensors were showed in Fig. 1. The effect of *R. mucilaginosa* modification on the electrode response for the determination of Cu(II) was clearly seen in Fig. 1(a). Well defined cathodic peak which belongs to copper was obtained at about 300 mV by modified CPE.

3.2. SEM characterization and elemental analysis of the developed microbial biosensor surface

The SEM micrograph of the microbial biosensor surface after loading of 1.0×10^{-3} M of Cu(II) were given in Fig. 2. The results of elemental analysis were also given in Table 1. Because of neither N nor Na was not found in the elemental analysis, the data verified that the round shapes which seemed on the SEM micrographs were belong to the oxidized copper ions.

3.3. The effect of scan rate

The effect of different scan rates on the electrode response for the voltammetric determination of 1.0×10^{-3} M Cu(II) with the *R. mucilaginosa* modified microbial biosensor was studied by varying

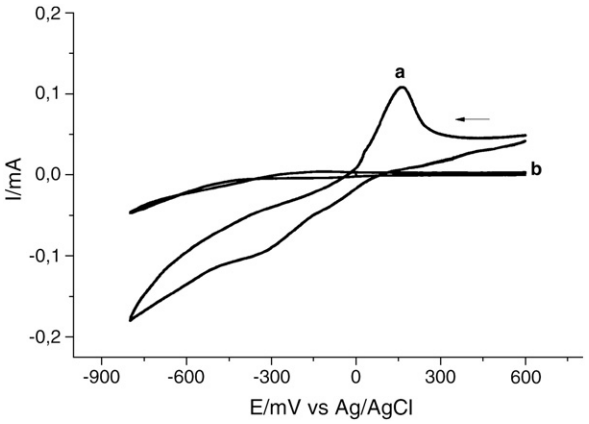

Fig. 1. Cyclic voltammograms of *R. mucilaginosa* modified CPE (a) and unmodified CPE (b). (Concentration of Cu(II): 1.0×10^{-3} M, detection: cyclic voltammetry in 0.05 M $NaNO_3$ with 100 mV/s scan rate, preconcentration time: 15 min).

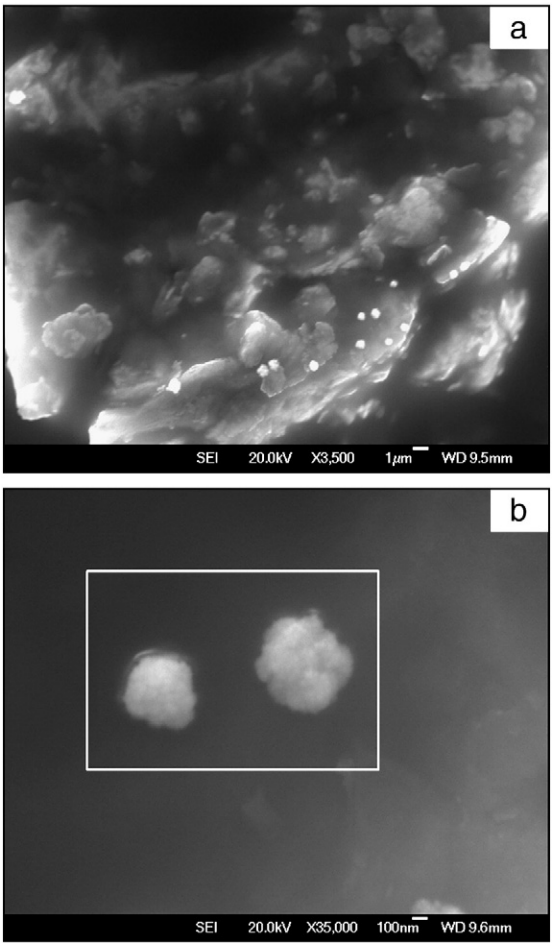

Fig. 2. SEM micrographs of the developed microbial biosensor after loading of copper onto the surface. Oxidized copper ions were clearly seen in (a) and (b).

scan rates between 40 and 120 mV/s. Biosensor response increased with an increase in the scan rate and became broader up to 100 mV/s. The higher scan rate caused a deviation on the peak shape and decreased peak current. The highest sensitivity was achieved at 100 mV/s scan rate and the other studies were performed at 100 mV/s. The effect of different scan rates on the microbial biosensor response was shown in Fig. 3.

3.4. The effect of preconcentration time

The length of time that the electrode is exposed to the analyte solution to allow transport of copper ions is an important factor. The effect of preconcentration time on the microbial biosensor response was investigated different time rates from 5 to 60 min. The preconcentration time increased the microbial biosensor response raised up to 60 min. However the peak current and shape belonging

Table 1
Elemental analysis results of the developed microbial biosensor.

After loading of Cu(II)	
Component	Conc. (wt.%)
Cu	5.521
C	85.986
O	8.492
N	0
Na	0

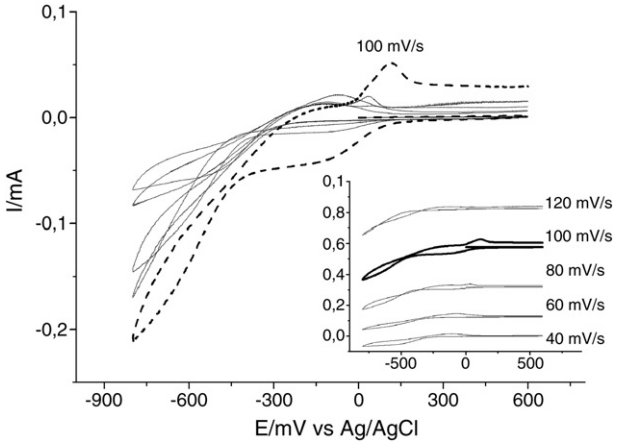

Fig. 3. Effect of different scan rates on the microbial biosensor response (Concentration of Cu(II): 1.0×10^{-3} M, detection: cyclic voltammetry in 0.05 M $NaNO_3$ with 100 mV/s scan rate, preconcentration time: 15 min).

to copper was decreased and changed after 15 min of preconcentration time (Fig. 4).

A conductivity test was also carried out to establish the optimum preconcentration time at open circuit potential (Fig. 5). According to the conductivity test results, maximum amount of Cu(II) was accumulated to biosensor surface in nearly 15 min with an increasing potential and after this time no alteration was observed at conductivity. Therefore 15 min preconcentration time was chosen as optimum accumulation time and used for later experiments.

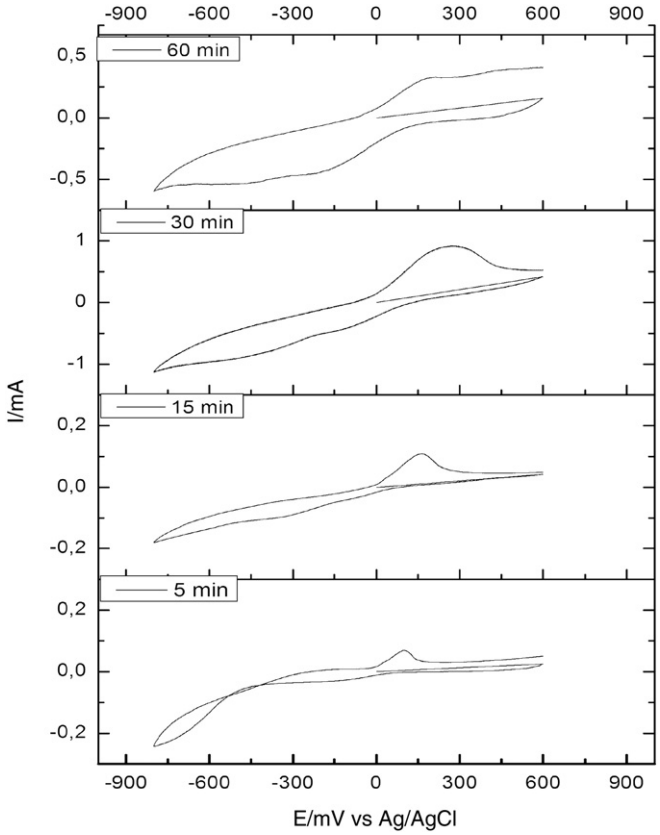

Fig. 4. Effect of preconcentration time on the microbial biosensor response (Concentration of Cu(II): 1.0×10^{-3} M, detection: cyclic voltammetry in 0.05 M $NaNO_3$ with 100 mV/s scan rate).

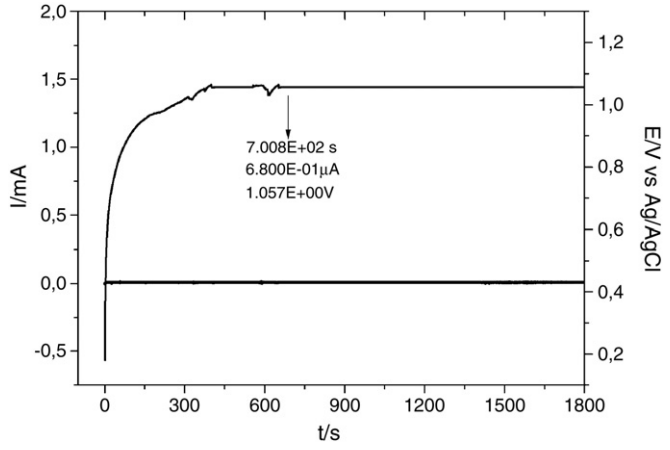

Fig. 5. Conductivity test result of the developed microbial biosensor (Concentration of Cu(II): 1.0×10^{-3} M, time: 1800 s, Conductivity test was carried out at open circuit potential).

3.5. The effect of preconcentration solution pH

The pH of preconcentration solution affects the solubility of metal ions and the ionization state of the functional groups of the yeast cell wall. According to the results of previously accomplished studies, copper belonging to the first class of metal ions that tightly and rapidly bound at pH greater than or equal to pH 5 and can be stripped at pH less than or equal to pH 2. Ions like Pb(II), Cd(II), Cr(III), Co(II), Ni(II), Zn(II), Fe(III) and UO_2(VI) also belong to this class. Biosorption of metal ions in this class appears to occur via an ion exchange process with metal cations competing with protons for negatively charged binding sites on the cell wall [11].

The effect of preconcentration solution pH on the microbial biosensor response was investigated from pH 4.0 to 8.0. Preconcentration solution pH levels greater than 6 caused precipitation of a large quantity of copper ions. Therefore, consistent results were not obtained at these pH levels. On the other hand, an acidic pH was resulted in an increase at peak current, because of protons' ability to displace copper from binding positions. The optimum copper accumulation with *R. mucilaginosa* modified microbial biosensor was occurred at pH 5 for 1.0×10^{-5} M Cu(II) (Fig. 6).

3.6. Analytical characteristics of developed microbial biosensor

The calibration plot obtained using the optimum conditions was depicted in Fig. 7. The peak current increased with increasing concentrations of Cu(II). The developed microbial biosensor was found to be a linear range between 1.0×10^{-7} and 1.0×10^{-5} M Cu(II) (0.0064 and 0.64 mg/L) (R^2: 0.98) for 15 min preconcentration time at open circuit. Deviation from the linearity occurred at higher concentrations of Cu(II) than 1.0×10^{-5} M may be due to saturation of the binding regions on the electrode surface. Amperometric detection of Cu(II) by *Saccharomyces cerevisiae* modified two biosensors using flow injection analysis (FIA) was reported before and linear ranges of both microbial biosensors were defined as 1.6–6.4 and 0.05–0.35 mg/L Cu(II) respectively [12]. Also a new system was reported for the amperometric detection of Cu(II) using recombinant *S. cerevisiae* strains as the biological material in the microbial biosensor application. The biosensor had a linear range between 5.0×10^{-4} and 2.10^{-3} M Cu(II) [13]. However our developed microbial biosensor is more sensitive and has a wider range for determination of copper ions.

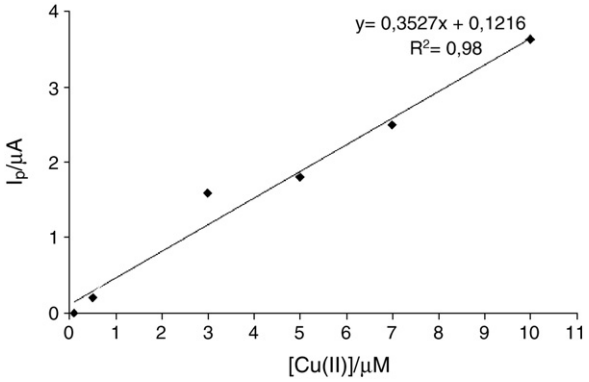

Fig. 7. Calibration plot of the developed microbial biosensor obtained under optimum experimental conditions.

When we compared with other properties for determination of Cu(II), our developed biosensor has some advantages about preparation and detection stages [12,13,15,16]. An Alg modified biosensor for determination of copper was reported before and response time was found 30 min [14]. Easy preparing, simple operating, short response time and low cost for fabrication were the most important features of the developed microbial biosensor. Table 2 shows that analytical and technique properties of some Cu(II) sensors and present developed microbial biosensor.

3.7. Single and matrix effects of the other heavy metal ions on the microbial biosensor response

The presence of other heavy metals either enhances or decreases the peak currents of a certain metal present in the analyte [18]. The response of microbial biosensor was evaluated in the presence of 1.0×10^{-6} M Cu(II) and the interfering metal ions, Ni(II), Zn(II) and Pb(II), at concentrations of between from 1.0×10^{-6} to 1.0×10^{-4} M and studies were conducted by Differential Pulse Stripping Voltammetry (DPSV). According to the results Zn(II) had no much influence on the biosensor response but, the other heavy metal ions decreased the peak current at high concentrations (Fig. 8). Ni(II) was decreased the most peak current of Cu(II). However at all studied concentrations of other heavy metals, the peak belonging to Cu(II) was observed clearly. More concentrations of other interferences just decreased peak current but not affected the determination of Cu(II) thoroughly.

The matrix effects of the interfering ions on the response of microbial biosensor were investigated using two different model solutions. First solution (Fig. 9(a)) contained of all heavy metals at equal concentrations (1.0×10^{-6} M of Cu(II), Ni(II), Zn(II) and Pb(II)) and the second solution (Fig. 9(b)) was arranged with 1.0×10^{-6} M Cu(II) and 5.0×10^{-6} M of the other heavy metals. According to the results, the peak current of Cu(II) was not affected by low concentrations of the other heavy

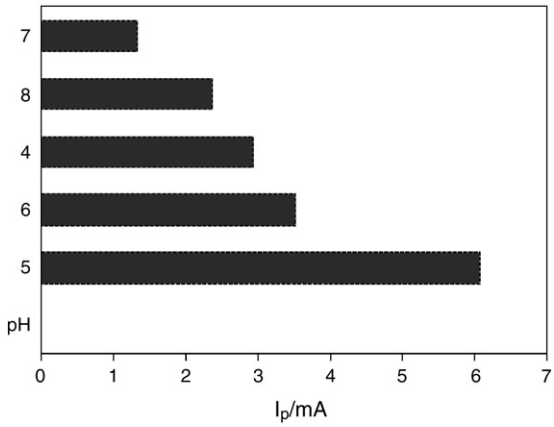

Fig. 6. Effect of preconcentration solution pH on the microbial biosensor response. (Concentration of Cu(II): 1.0×10^{-5} M, detection: DPSV in 0.05 M $NaNO_3$, preconcentration time: 15 min).

Table 2
Analytical and technique properties of some Cu(II) sensors.

Agent	Preparation	Detection	Time (min)	Linear range	Ref.
Recombinant *Saccharomyces cerevisiae*	Immobilization of the cells on a capillary membrane	Flow injection analysis	>30	0.05–0.35 mg/L	[12]
Recombinant *Saccharomyces cerevisiae*	Immobilization of the cells on a capillary membrane	Amperometric	>20	5.0×10^{-4}–2.0×10^{-3} M	[13]
Circinella sp.	Incorporating non-living biomass in carbon paste	Stripping voltammetry	30	5.0×10^{-7}–1.0×10^{-5} M	[14]
α-Benzoinoxime	Immobilization of α-Benzoinoxime on XAD-2	Gravimetric colorimetric	4	5.0–127 mg/L	[15]
A schiff base	Immobilization of schiff base on polyvinlyl chloride	Optical	2.5	1.0×10^{-8}–5.7×10^{-4} M	[16]
Natural zeolite	Incorporating non-living biomass in carbon paste	Stripping voltammetry	15	5.0×10^{-8}–5.0×10^{-6} M	[17]
Lyophilized *Rhodotorula mucilaginosa*	Incorporating non-living biomass in carbon paste	Stripping voltammetry	15	1.0×10^{-7}–1.0×10^{-5} M	[a]

[a] Present work.

metals. Although, the peak current of Cu(II) showed a decrease at higher concentration of interfering metal ions, obtained peaks demonstrated that Cu(II) was reliably distinguished from all these heavy metals at optimum experimental conditions.

4. Conclusion

In the present study, a new microbial biosensor was developed using *R. mucilaginosa* as a sensor modifying biological agent for the determination of Cu(II) at trace levels by Differential Pulse Stripping Voltammetry. Compared with other stripping voltammetric determinations, the method presented here has a lower detection limit [12,13,19]. The developed microbial biosensor is also simple to prepare, a low cost and does not require extensive preliminary sample treatment and is not interfered so strongly by interfering heavy metals, such as Pb(II), Zn(II) which are normally associated with copper in nature.

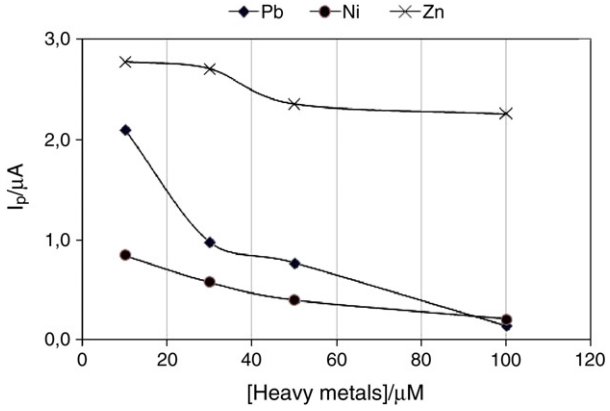

Fig. 8. Single effects of the other heavy metal ions on the microbial biosensor response for copper determination (Concentration of Cu(II): 1.0×10^{-6} M and the other heavy metal ions, Ni(II), Zn(II) and Pb(II) at concentrations of 1.0×10^{-6} to 1.0×10^{-4} M, detection: DPSV in 0.05 M $NaNO_3$, pH:5, preconcentration time: 15 min).

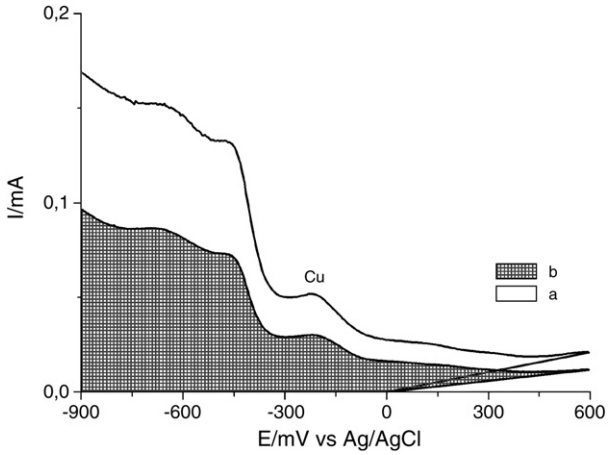

Fig. 9. Matrix effects of the other heavy metal ions on the microbial biosensor response, voltammograms were obtained using two different metal ion mixtures including Cu(II), Ni(II), Zn(II) and Pb(II), (a: the mixture of , 1.0×10^{-6} M of all the metals b: the mixture of 5.0×10^{-6} M of Ni(II), Zn(II), Pb(II) and 1.0×10^{-6} M of Cu(II); detection: DPSV in 0.05 M $NaNO_3$, pH:5, preconcentration time: 15 min).

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