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Utilization of heat-dried *Pseudomonas aeruginosa* biomass for voltammetric determination of Pb(II)

Meral Yüce[1], Hasan Nazır[2] and Gönül Dönmez[3,]

[1] Ankara University, Institute of Biotechnology, 06100 Beşevler, Ankara, Turkey
[2] Faculty of Science, Department of Chemistry 06100 Beşevler, Ankara, Turkey
[3] Faculty of Science, Department of Biology, 06100 Beşevler, Ankara, Turkey

In this research, thermally dried *Pseudomonas aeruginosa* cells were used as a biological material for the construction of a microbial biosensor. The preparation, optimization and application of the developed microbial biosensor, which analyzed Pb(II), are presented. The method was based on stripping of adsorbed metal ions from the modified electrode surface. Modified carbon paste electrodes were preconcentrated at open circuit, and then electrochemically measured by using cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPSV) techniques. It was found that the thermally dried cells were capable of adsorbing Pb(II) ions from aqueous solutions and could determine the ions prominently at optimum experimental conditions. Many important parameters to acquire the best electrochemical response were carried out, including effect of different electrolyte solutions, pH, deposition potential, deposition time, ionic strength, preconcentration time, and effect of interference ions. Finally, a calibration graph was obtained with a linear range from 1.0×10^{-6} to 2.0×10^{-5} M Pb(II) ($R^2 = 0.9916$) and detection limit was found as 6.0×10^{-7} M Pb(II) by using $3 \times S_b/m$ formula. Other analytical properties of the developed microbial biosensor were also investigated. The suggested usage format of *P. aeruginosa* for the determination of Pb(II) does not require complicated immobilization procedure, easy to handle, and not time consuming.

Introduction

Determination and removal of heavy metal ions are of environmental concern because of their toxic effects on the living organisms, including humans [1]. Among these heavy metals, Pb(II) which is the eighty second element of the periodic table is like other heavy metals strongly harmful to organisms even at very low levels, leading to respiratory and gastric troubles [2–4].

Biosensors have been used in the determination of several heavy metal species including Cu(II), Cr(VI), Pb(II) and Ni(II) [5–9]. It has been known since the sixties that microorganisms adsorb or bioaccumulate a wide range of chemical substances through their biosorption capability. Application of microorganisms as sensor modifying agents has important advantages, for example it is simple, economical, and suitable to product at large scale, and therefore has received considerable attention in recent years [10,11]. Many microorganisms, including bacteria, algae, and fungi, have been reported that are sensitive to specific metals and can determine the heavy metals reliably from aqueous solutions [12–16].

Generally, passive uptake of heavy metals by using dead biomass is more efficient, economical, and easier than active uptake by using living biomass because it requires no nutrients, is easy to handle and store, low cost and has high tolerance to over concentration of toxic substances [17,18]. In addition to these, heat pretreatment reveals secondary or latent binding sites of the biomass, providing in an increase in the total uptake [19]. The Pb(II) binding is thought to occur mainly on the negatively charged carboxylate groups on the cell wall [20].

Corresponding author: Dönmez, G. (gdonmez@science.ankara.edu.tr)

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Specific metal binding properties of certain types of nonliving biomass have been combined with electrochemical techniques for the construction of microbial ion selective sensors. Among these studies, there were a few about the usage of heat-dried cells as a biological material for the construction of microbial sensors to determine heavy metal ions selectively [1,21,22].

In the present study, *Pseudomonas aeruginosa*, which is a gram negative, aerobic rod belonging to the bacterial family Pseudomonadaceae, was used in heat dried form as a biological material to construct a microbial biosensor detecting Pb(II) ions from aqueous solutions prominently. Many important electrochemical parameters were performed to reach the best sensitivity. Although *P. aeruginosa* has been widely used in the heavy metal biosorption studies [2,23,24], application of heat dried form of *P. aeruginosa* determinining Pb(II) electrochemically from aqueous solutions has not been reported as far as we know. The proposed method was found selective, economic and not time consuming for Pb(II) determination by means of usage of heat-dried *P. aeruginosa* cells.

Experimental

Chemicals

All solutions were prepared with distilled water. Stock solutions of Co(II), Cu(II), Ni(II), Zn(II), and Pb(II) (1.0×10^{-1} M) were prepared from the corresponding analytical grade metal nitrates (Merck, Darmstadt, Germany). Working solutions of metal ions were prepared freshly from stock solutions after appropriate dilutions with distilled water. Sodium acetate buffer was used as a main supporting electrolyte solution, and was prepared by mixing a certain amount of glacial acetic acid (Carlo Elba, Italy) with sodium acetate (Merck, Darmstadt, Germany). Graphite powder and paraffin liquid (Merck, Darmstadt, Germany) were used for preparing the carbon paste electrode (CPE). Nutrient Broth medium was used for cultivation of the microorganism.

Microorganism and culture conditions

P. aeruginosa used as a biological material to modify CPE was obtained from Culture Collection of Biotechnology Research Laboratory, Biology Department, Ankara University, Turkey.

Nutrient Broth medium was used for cultivation. The microorganism was incubated for three days at 35 °C in a rotary shaker at 120 rpm (New Brunswick Scientific Innova 4230). After the incubation period *P. aeruginosa* was harvested by centrifugation at 10 000 rpm for 5 min, the supernatant was discarded and the pellet washed twice with distilled water. The obtained biomass was dried at 70 °C for 36 hours in glass plates. Finally, heat-dried biomass was removed by scratching, and powdered by using a ceramic mortar.

Operating principle of the developed microbial biosensor

The modified CPE was prepared by making a homogeneous paste with 5.0 mg of heat-dried nonliving cells, 95 mg of graphite powder and a proper amount of paraffin liquid. The paste mixture was firmly packed into the hole of the homemade electrode body (5.0 mm diameter), and was compressed with a steel rod. Then the electrode surface was smoothed on paper. After preconcentration step, the electrode was immersed into 20 ml of sodium acetate solution, and was measured electrochemically.

Apparatus and experimental method

Electrochemical experiments were performed at room temperature with a CH Instrument 660B model Electrochemical Analyzer (CH Instrument, USA). A conventional three-electrode cell configuration was employed throughout experiments, consisting of a homemade CPE modified with heat-dried *P. aeruginosa* cells; a platinum wire counter electrode and an Ag/AgCl reference electrode (Saturated KCl). The electrode cell containing supporting electrolyte solution, 0.05 M sodium acetate buffer, was deoxygenated by passing pure nitrogen through it before each measurement.

Experiments were done using the accumulation, medium change, and voltammetry scheme. A 5.0 mg of heat-dried biomass was used for making 5% (w/w) carbon pastes. In the preconcentration step, the modified CPE was immersed in 20 ml of stirred metal ion solution for a selected time at open circuit. After this step, the biosensor was taken out and its surface was washed carefully with distilled water. Finally, the biosensor was placed in the electrochemical cell filled with 20 ml of 0.05 M sodium acetate solution, connected to the device, and voltammetrically measured. Cyclic voltammetry and differential pulse stripping voltammetry assays were performed with modified CPEs, which were preconcentrated in Pb(II) solutions. The voltammograms covered from −1.5 to 1.5 V potential range and swept back for CV assays versus the Ag/AgCl reference electrode. The background current was also obtained after the accumulation step in the absence of Pb(II) blank solution.

Results and discussion

Cyclic voltammetry assays

The cyclic behaviors of the modified and nonmodified CPEs were obtained in 0.05 M sodium acetate buffer for 1.0×10^{-4} M Pb(II) and are shown in Fig. 1. The cyclic behavior of *P. aeruginosa* modified biosensor distinguished from that of unmodified electrode clearly at the potential value of about −0.5 V (Fig. 1a). A well defined peak belonging to Pb(II) depicted the capability of the biological material to adsorb Pb(II) effectively. This potential value at which Pb(II) peak occurred on the voltammogram was in agreement with the results obtained by other researchers [25,26].

Supporting electrolyte solutions, pH and ionic strength

Different electrolyte solutions containing 1.0×10^{-4} M Pb(II) were tested to catch oxidation or reduction peaks belong to Pb(II) by using CV technique: 0.05 M of $NaNO_3$, Tris–HCl buffer, NH_4Cl buffer and CH_3COONa buffer. Among all these solutions, the peak indicated presence of Pb(II) on the modified electrode surface was just monitored when the sodium acetate buffer was used as a supporting electrolyte solution.

The pH of the electrolyte solution strongly influences the biosorption availability of the metal ions and the activity of the functional groups binding metals. At high pH values, metal ions in the solution undergo hydrolysis and the solubility of the metal ions decreases allowing the precipitation to affect the biosorption process negatively [18]. A simple chemical equation scheme showing hydrolysis of most divalent metal ions is given below [27]:

$$M_{(aq)}{}^{2+} + H_2O \rightleftharpoons M(OH)_{(aq)}{}^{+} + H^{+} \quad (1)$$

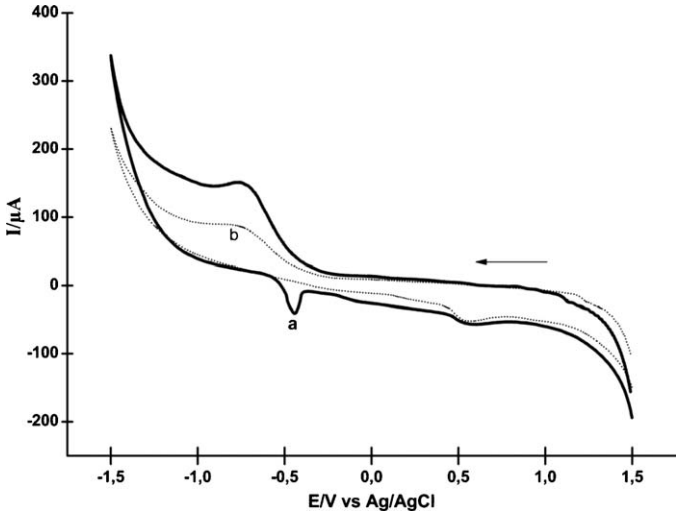

FIG. 1

Cyclic voltammograms of *P. aeruginosa* modified CPE (a) and unmodified CPE (b). (Concentration of Pb(II): 1.0×10^{-4} M, detection: CV in 0.05 M sodium acetate buffer.)

where M^{2+} is either Pb(II) or another divalent metal ion. The retention of metals on to the electrode surface decreases at high pH levels because of the formation of hydroxylated complexes. By contrast, negatively charged functional groups on the biomass surface, which are responsible for binding of metal cations, are protonated at very low pH values because of hydronium ions. Consequently, adsorption ability of the metals to electrode surface decreases in acidic solution [28]. We tested electrochemical responses in a pH range from 3.5 to 6.0, and found the best sensitivity at pH 5.0 for 1.0×10^{-5} M Pb(II) as shown in Fig. 2.

Other investigators [29–31] also marked acidic pH values for Pb(II) determination. The peak current belonging to Pb(II) increased up to pH 5.0. This may be due to the dissociation of hydronium ions from functional sites of the biomass surface that means there are more binding sites for metal ions. pH values higher than 5.0 caused hydrolysis, the peak current decreased as a result of precipitation of metal ions in the solution.

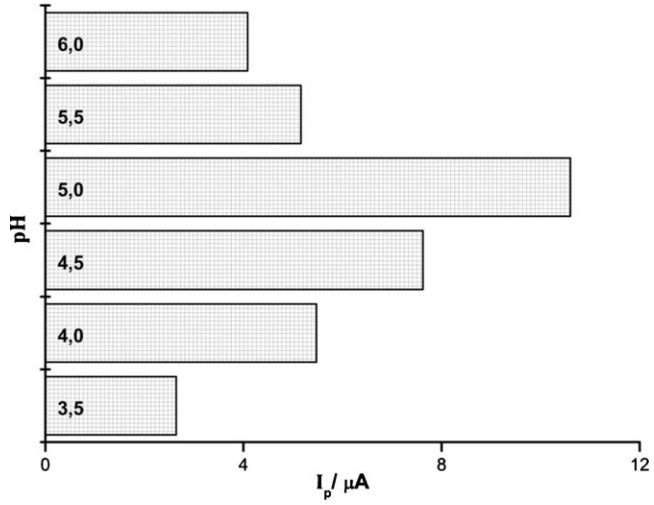

FIG. 2

Effect of solution pH on the peak current. (Concentration of Pb(II): 1.0×10^{-5} M, detection: DPSV in 0.05 M sodium acetate buffer.)

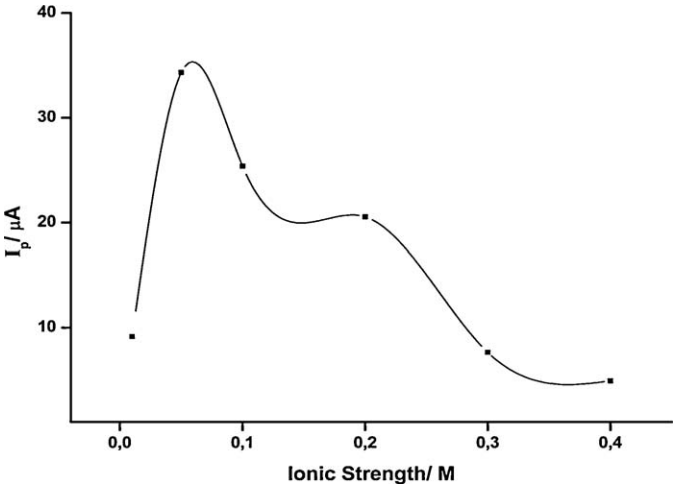

FIG. 3

Effect of ionic strength on the peak current. (Concentration of Pb(II): 2.0×10^{-5} M, detection: DPSV in 0.05 M sodium acetate buffer.)

Ionic strength is also an important parameter owing to the fact that concentration of supporting electrolyte solution affects the solubility of the metal ions and hence adsorption period. Previous studies showed that adsorption decreases with an increase in ionic strength [32,33]. Therefore, we tested this parameter with six concentrations of sodium acetate buffer (0.01, 0.05, 0.1, 0.2, 0.3 and 0.4 M) for 2.0×10^{-5} M Pb(II). According to the obtained results, the maximum peak current occurred when the concentration of solution was 0.05 M. The peak current after this point decreased gradually as presented in Fig. 3. The decline at high concentrations of electrolyte solution may be explained by the competition between Pb(II) ions and protons for binding sites, or the formation of weak lead acetate complexes lowering the concentration of Pb(II) in solution, and therefore biosorption of it.

The effect of preconcentration time

Determination of preconcentration time has a significant effect on the size of the stripping peak. Peak size, and hence the peak current increase when the preconcentration time increases, in accordance with the saturation of functional sites on the biological material. Thus, a time range between from 2.0 to 16 min was operated for 2.0×10^{-5} M Pb(II). The increase of preconcentration time concluded an increment in the peak current up to 12 min. At longer preconcentration times, relevant peak current started to reduce as shown in Fig. 4. This decrease may be explained by the saturation of binding sites on the electrode surface or equilibrium state between the bonded Pb(II) ions and the ions in solution [18]. Consequently, a preconcentration time of 12 min was selected as an optimum accumulation time, and was applied for further studies.

The effect of the applied deposition potential and deposition time

The period spent for reducing Pb(II) ions to their metallic form is described as deposition time, and deposition potential is needed to reduce or deposit adsorbed ions from the electrode surface [30]. In this study, a deposition potential range from −1.6 to −0.4 V was investigated as presented in Fig. 5, the peak shape improved and peak current rose up to −1.4 V, but a sharp decrease was observed

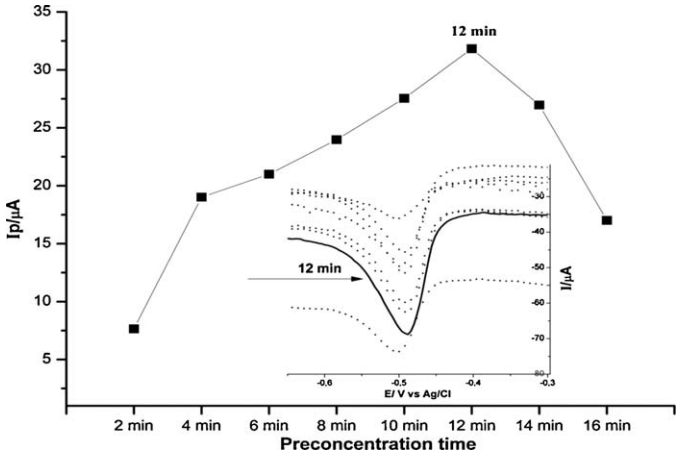

FIG. 4

Effect of preconcentration time on the microbial biosensor response. (Concentration of Pb(II): 2.0×10^{-5} M, detection: DPSV in 0.05 M sodium acetate buffer.)

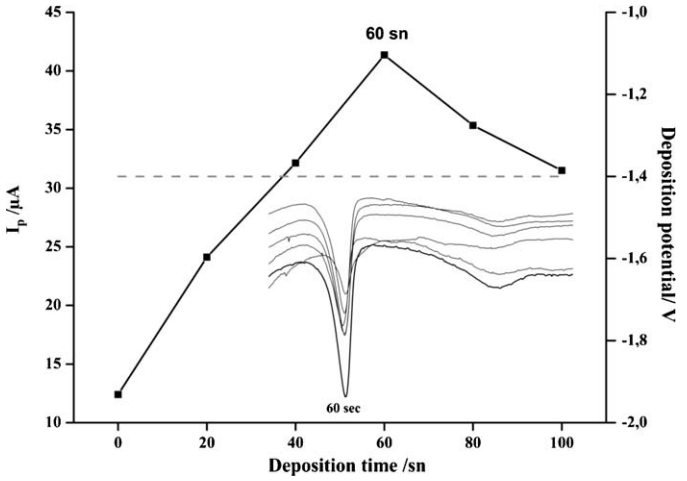

FIG. 6

Effect of deposition time on the peak current. (Concentration of Pb(II): 2.0×10^{-5} M, deposition potential: −1.4 V, preconcentration time at open circuit: 12 min, detection: DPSV in 0.05 M sodium acetate buffer.)

at more negative potentials and background current gradually deteriorated. Previous studies had also verified that Pb(II) strongly stripped at more negative potentials [34].

We continued this study by investigating the effect of deposition time on the stripping signals and performed a series of experiments to detect optimum deposition time for ensuring the best stripping peak. Deposition time was studied between 0.0 and 100 s with a constant deposition potential of −1.4 V for 2.0×10^{-5} M Pb(II), and maximum peak current occurred when the 60 s deposition time was applied (Fig. 6).

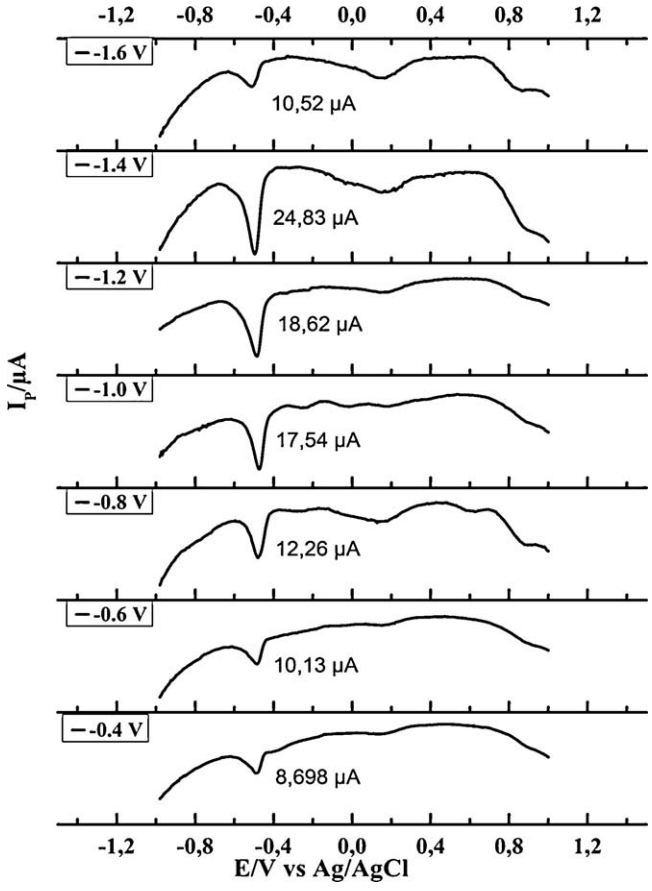

FIG. 5

Effect of deposition potential on the peak current. (Concentration of Pb(II): 2.0×10^{-5} M, preconcentration time at open circuit: 12 min, detection: DPSV in 0.05 M sodium acetate buffer.)

Analytical properties of the developed microbial biosensor

The calibration graph obtained using the optimum experimental conditions (electrolyte solution: 0.05 M sodium acetate buffer, pH: 5.0, preconcentration time at open circuit: 12 min, deposition potential: −1.4 V, deposition time: 60 s) was defined by the equation $y = 1.6283x - 0.6058$ and is depicted in Fig. 7 together with other analytical properties of the developed sensor. The peak current belonging to Pb(II) (y) was correlated with Pb(II) concentration (x). It was linear from 1.0×10^{-6} M to 2.0×10^{-5} M Pb(II) ($n = 2$, $R^2 = 0.9916$) and above 1.0×10^{-6} M Pb(II), the calibration curve tended to level off because of the saturation of electrode surface. Limit of detection was calculated as 6.0×10^{-7} M by using

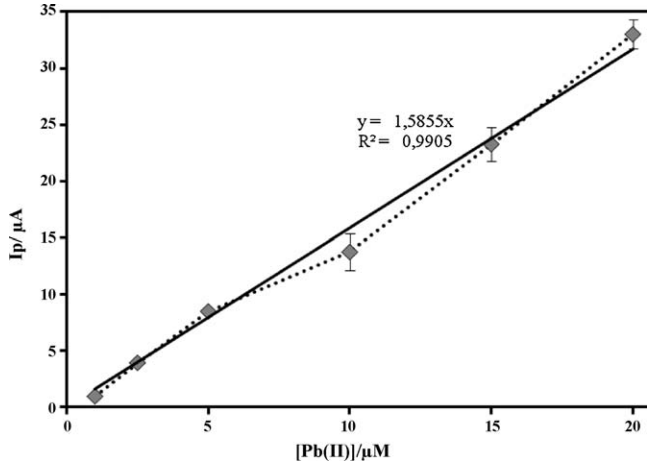

FIG. 7

Calibration curve and some analytical properties of the developed microbial sensor. (Electrolyte solution: 0.05 M of sodium acetate buffer, pH: 5.0, preconcentration time at open circuit: 12 min, deposition potential: −1.4 V, deposition time: 60 s.)

TABLE 1

Analytical and technique properties of some Pb(II) sensors.

Agent	Preparation	Detection	Time	Linear range	Ref.
Banana tissue	Incorporating banana tissue in carbon paste	Stripping voltammetry	6 min	1.0–20 ppm	[30]
Pennisetum setosum	Incorporating treated and nontreated biomass in carbon paste	Stripping voltammetry	5 min	0.02–0.08 ppm	[32]
Bovine serum albumin (BSA)	Incorporating nonliving biomass in carbon paste	Stripping voltammetry	>2 hours	4.0×10^{-3}–6.0×10^{-5} ppm	[9]
$HCLO_4$	Incorporating conducting binder in carbon paste	Linear sweep voltammetry	15 min	0.1–2.0 ppm	[33]
Orange peel essential oil	Immobilization of Schiff base on polyvinlyl chloride	Stripping voltammetry	2.5 min	1.0–25 ppm	[36]
***Phormidium* sp.**	Incorporating modified biomass in carbon paste	Stripping voltammetry	10 min	0.01–4.0 ppm	[43]
Heat-dried *P. aeruginosa*	Incorporating nonliving biomass in carbon paste	Stripping voltammetry	12 min	0.2–4.0 ppm	Present study

TABLE 2

Effect of interference ions on the electrochemical response in the presence of Pb(II).

Interferences	[Metals] (μM)	I_p (μA)	% activity
Pb(II)	10	12.59 ± 0.02	100
Co(II)	10	0	0
Pb(II) + Co(II)	10 + 10	13.70 ± 0.29	109
Cu(II)		10	0
Pb(II) + Cu(II)	10 + 10	8.91 ± 0.14	71
Ni(II)		10	0
Pb(II) + Ni(II)	10 + 10	3.20 ± 0.09	25
Zn(II)		10	0
Pb(II) + Zn(II)	10 + 10	18.62 ± 1.48	148
Pb(II) + Zn(II) + Cu(II) + Co(II) + Ni(II)	10 + 10 + 10 + 10 + 10	7.44 ± 0.04	59
Pb(II) + Zn(II) + Cu(II) + Co(II) + Ni(II)	10 + 30 + 30 + 30 + 30	2.71 ± 0.41	22

All analysis were performed two times and reported as average ± standard deviation.

$3S_b/m$ formula [35]. A $HClO_4$ modified CPE and a *Pennisetum setosum* modified CPE were reported previously for Pb(II) determination that both had tighter linear ranges than our developed biosensor [31,32]. Table 1 shows analytical and technique properties of some Pb(II) sensors and presents developed microbial biosensor.

The reproducibility of the developed microbiosensor was also tested for 1.5×10^{-5} M Pb(II) ($n = 7$). According to the results obtained, the mean of current values were found to be 25.63 μA with a standard deviation of 1.06, and a variation coefficient (CV) was 4%.

Effect of interference ions on the electrochemical response in the presence of Pb(II)

The influences of some ions, which are known as interference ions, on the electrode response were investigated in the optimum experimental conditions, on the ground that the presence of other heavy metals in the media either enhances or decreases the peak currents of a certain metal present in the analyte [37]. For this reason, Pb(II) was added to solutions with a constant concentration (1.0×10^{-5} M), and single, binary and multi effects of the other heavy metals [Co(II), Cu(II), Ni(II) and Zn(II)] on the electrode response were investigated by using DPSV technique. The results are summarized in Table 2. According to the data obtained, none of the interference metal ions gave traceable peaks when they were singly in the solution, whereas they affected the response in a way of positive or negative when they were in binary form with Pb(II) in the solution. The presence of Cu(II) and Ni(II) in the solution as binary or as a mixture caused an antagonistic effect with a value of about 30% on the Pb(II) determination. By contrast, Zn(II) showed a strong synergistic effect and enhanced the electrochemical response. Cu(II) showed also a synergistic effect, but less than that Pb(II) showed.

When all metals were in the solution in equal concentrations, peak current belonging to Pb(II) reduced to nearly half. However, relevant stripping peak was still observable and quantifiable even in the presence of extreme concentrations of the other heavy metals.

Conclusion

The ultimate aim of this study is to search if heat-dried *P. aeruginosa* cells, which is low cost, easy to cultivate and requiring minimal pretreatment, serve as a biomaterial for the determination of heavy metals from aqueous solutions by using CPEs or not. We performed various assays and brought to light that CPE mod-

ified with heat-dried *P. aeruginosa* was capable of detecting Pb(II) selectively from aqueous solutions containing interference metal ions in different concentrations.

Some investigators have used *P. aeruginosa* up to now in biosensor constructions [38–41], and metal biosorption studies [42]. However, heat-dried *P. aeruginosa* cells have not been studied yet with aim and method mentioned above, thus the present investigation has revealed a simple usage format for *P. aeruginosa* biomass.

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