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***Escherichia coli*-functionalized magnetic nanobeads as an ultrasensitive biosensor for heavy metals**

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Abstract

The detection of traces of heavy metals in real environmental samples is a difficult task. In this work, we combined the advantages of nanobeads' (NBs) properties with the simplicity of the layer-by-layer procedure, along with the use of micro-organisms as bioreceptors, for the elaboration of a novel electrochemical biosensor based on nanocomposite films. The whole cells (*Escherichia coli*) K-12 used as bioreceptors were fixed onto a surface of indium-tin-oxide (ITO) glass with and without NBs and/or polyelectrolyte multilayers (PEM). Using the electrochemical impedance spectroscopy (EIS) technique, cadmium and mercury were detected.

Keywords: Biosensor, nanobeads, PEM, heavy metals, *Escherichia Coli*, EIS

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1. Introduction

Heavy metals are dangerous to human health when present in drinking water and food [1]. The main restrictions result from the detection of their extremely low concentrations. Many techniques are used for heavy metals quantization but are often complex and expensive. In recent years, the development of whole-cell biosensors has been met with an increasing interest [2,3].

In this work, we combined the advantages of nanobead properties, the layer- by -layer simplicity procedure and micro-organisms to elaborate an electrochemical biosensor for heavy metal detection. This biosensor is based on whole cells immobilized on nanocomposite film which is adsorbed onto indium-tin-oxide (ITO) glass as work electrode. The proposed biosensor exhibits fast and sensitive electrochemical responses to cadmium and mercury. The biosensor's analytical performances were optimized by comparing four membranes elaborated on the work electrode surfaces : bacteria are deposited directly on the electrode surface (ITO-bacteria), bacteria deposited on the polyelectrolyte multilayers functionalized electrode (ITO-(PEM)₃-bacteria), bacteria deposited on nanobeads electrode surface (ITO-NBs-bacteria), and bacteria deposited on NBs functionalized PEM electrode surface (ITO-NBs-(PEM)₃-bacteria).

2. Materials and methods

2.1 Chemicals

Magnetic nanobeads used in this work are ferric oxide (Fe₂O₃) from Sigma Aldrich compagny (Iron Oxide nanopowder 98%). The nanobead sizes were in the 10-20 nm range. These nanobeads were suspended in ultrapure water (milliQ) with a resistance about 18.0 MΩ.cm⁻¹, initially filtered through a 0.45 μm millipore filter, to obtain a 1 % w/w stock suspension.

Polyelectrolytes (PE) used are poly(allylamine hydrochlorure) (PAH, cation) and poly(styrene sulfonate) (PSS, anion) from Aldrich. The LBL deposition method results in self-assembled molecular multilayers (PAH-PSS)_n. Typically, it consists in alternated immersion of pretreated substrates during 20 minutes in PAH and PSS solutions (5 mg/ml in 0.15 M NaCl). After each step, substrates are rinsed with 0.15 M NaCl. For a typical three-bilayers, the entire coating takes 2 to 3 hours.

Stock solutions (1g.L⁻¹) of Cd²⁺ and Hg²⁺ were prepared from Cd(NO₃)₂·4H₂O and Hg(NO₃)₂·H₂O in PBS purchased at ACROS ORGANICS.

2.2 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy is an effective approach to quantify the electron-transfer resistance (R_{et}) of the electrochemical reactions produced at metal-electrolyte interfaces [4]. As it is sensitive to surface phenomena and changes of bulk properties, this method is particularly suited to the detection of binding events.

The electrochemical system consists of three electrode-electrochemical cell and a potentiostat connected to a PC computer. The used electrodes are the working, the reference and the auxiliary or the counter electrode. The reference electrode is a saturated calomel electrode Hg/Hg₂Cl₂/KCl (sat) one. The counter electrode is a platinum metal (99% purity) while the working electrode is a semiconducting indium-tin-oxide (ITO) glass. Before using the ITO electrodes, they were carefully cleaned by successive ultra sonications in different solvents. Firstly, the electrodes were sonicated in acetone (99.99 %) for 15 min then in ethanol for 15 min. Each sonication step was followed by rinsing the samples two or three times with H₂O and dried with nitrogen flow.

The method consists in applying to the electrochemical system under study a sinusoidal AC interfacial potential $V(\omega, t)$ and measuring the resulting current response $I(\omega, t)$ flowing through the system. The ratio of the applied voltage to the measured current is the impedance of the system which is easily calculated over a wide frequency range (1). The impedance spectra are obtained by sequential measurements of $V(\omega, t)$ and $I(\omega, t)$ for each single frequency that contributes to the spectrum.

$$Z = Z_{real} + j Z_{im} \quad (1)$$

The measurement data can be presented in Nyquist plot in which the imaginary impedance $Z_{im}(\omega)$ is plotted versus the real impedance $Z_{real}(\omega)$.

The impedance spectra were realized for thin polyelectrolyte multilayer films (PEM) deposited on the ITO working electrode. They were prepared by means of alternate adsorption of (PAH-PSS) bilayers, as previously described [5]. The buildup of films was 3 bilayers, called $(PEM)_3$ $(PEM)_i = (PAH-PSS)_i$.

3. Results and discussion

3.1 Impedimetric monitoring of bacteria deposited on ITO-bacteria and ITO-NBs-bacteria

The impedance spectra realized with ITO and ITO-NBs bacteria are illustrated in Figure 1A and 1B respectively for the detection of cadmium metal. *E. Coli* induce an increase of the electron transfer resistance (R_{et}) at the transducer surface with or without NBs. The results showed clearly that using immobilized bacteria on ITO without nanobeads (ITO-bacteria), the detection limit was 10^{-5} M for cadmium metal (Figure 1A). Compared to bacteria immobilized on ITO with nanobeads (ITO -NBs-bacteria), this detection limit was 10^{-12} M for cadmium (Figure 1B). The same results were obtained with mercury (Figures not shown).

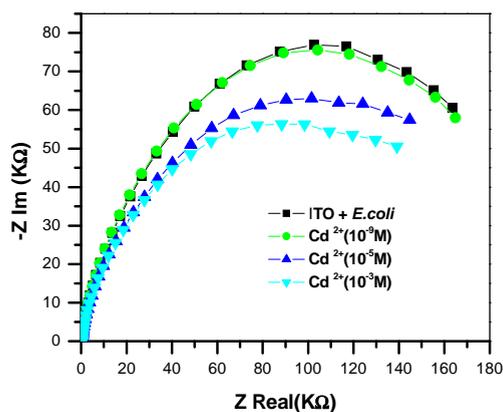


Figure 1A

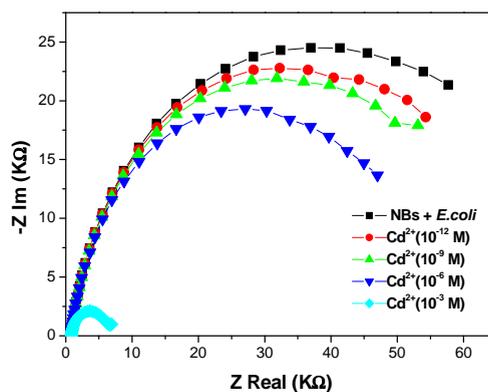


Figure 1B

Figure 1: Nyquist diagram (Z_{real} vs $-Z_{im}$) of electrochemical impedance spectra for the cadmium detection (Cd^{2+}) with two electrodes : (ITO-bacteria) (1A) and (ITO-NBs-bacteria) (1B).

It can be seen clearly that heavy metals as inhibitors of *E.coli* enzyme biosynthesis [6]. They interact with the membrane constituents and induce a decrease of its viability.

The process of the enzymatic inhibition is well described as based on the interaction between heavy metal and the thiol groups present in the cystein side chains.

3.2 Impedimetric monitoring of bacteria deposited on ITO-(PEM)₃-bacteria and ITO-NBs-(PEM)₃-bacteria.

On the other hand, bacteria were immobilized using polyelectrolytes with or without nanobeads indicated respectively by (ITO-NBs-(PEM)₃-bacteria) and (ITO-(PEM)₃-bacteria). Similarly as above, concentration range tested for Hg^{2+} ions varies from 10^{-12} to 10^{-3} M (Figure 2). The detection limit was 10^{-12} M for the two systems. However, the recorded signal is higher with bacteria immobilized on PEM functionalized NBs (Figure 2B) than with PEM adsorbed on ITO surface (Figure 2A). The same results were obtained with Cadmium metal (Figure not shown).

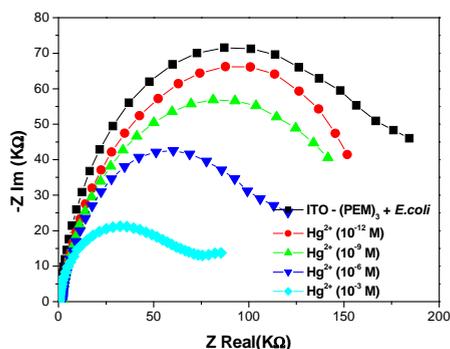


Figure 2A

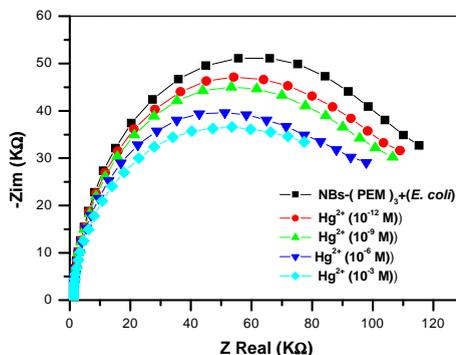


Figure 2B

Figure 2: Nyquist diagram (Z_{real} vs $-Z_{\text{im}}$) of electrochemical impedance spectra for the mercury detection (Hg^{2+}) with two electrodes: [ITO-(PEM)₃-bacteria] (2A) and [ITO-NBs-(PEM)₃-bacteria] (2B).

4. Conclusion

Finally in this work, we have developed a cell biosensor based on the immobilization of bacteria *E.coli* on Fe_2O_3 magnetic nanobeads with or without polyelectrolyte multilayers for the detection of heavy metals, particularly cadmium and mercury. The nanobiocomposite film provided a suitable microenvironment, which could effectively present large bacteria loading capacity and prevent the leaching out of the immobilized bacteria. The resulted biosensor exhibited a good analytical performance for the electrochemical detection of heavy metals and showed high sensitivity, low detection limit and very good reproducibility using the nanobeads system compared to established conventional methods. Furthermore, the method described here is all the more promising as it is versatile and not costly. However, the response of the proposed strategy remained unspecific. It could be extended to the development of genetically modified bacteria or microorganism-based biosensor systems for a specific and multi-detection of a wider range of heavy metals or other toxic substances.

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