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Enchanced Lead Ions Detection on Bacteria Modified Boron-doped Diamond Electrode

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Abstract. The wide electrochemical potential window of CVD diamond allows the cathodic detection of Pb^{2+} ions at voltages that lie outside the electrochemical potential window of conventional metal electrodes. The immobilization of bacterial cells, *Acidithiobacilllus ferrooxidans* on the surface of diamond enhances the detection limit for Pb^{2+} ions by two fold, which is 10 μM. The bacteria-modified diamond electrode provides linear detection range for Pb^{2+} from 10 μM up to 200 μM and higher sensitivity within the linear range. The unique property of *Acidithiobacilllus ferrooxidans* in fixing metal ions within its membrane provides the preconcentration effect needed in stripping voltammetry.

Keywords: electrochemical, bacterial cells, diamond.

1. Introduction

Boron-doped diamond is an attractive candidate for electroanalysis owing to its wide electrochemical window[1], low background current[2], chemical inertness[3] and high stability[4]. Fujishima *et. al.* demonstrated the use of diamond electrode in the stripping voltammetry of metal ions[5]. In stripping voltammetry, the electrode is initially held at a fixed potential in order to electrochemically deposit the analyte of interest onto the electrode surface. This is also known as the preconcentration step. The electrode will then be scanned either anodically or cathodically in order to liberate the analyte as an ion from the electrode. This step results in a faradaic current flow which can be used for the direct quantification for the analyte. Traditionally, stripping voltammetry is performed with a hanging mercury drop as a mercury amalgam will be formed upon deposition of metal ions in the preconcentration step. However, the toxicity and disposal problem of mercury has motivated the development of mercury-free electrode. Comparison between boron-doped diamond electrode and mercury electrode shows that diamond provides at least three times improvement in sensitivity[6]. Various methods such as microwave enhanced stripping voltammetry[7], laser activation[8], sonoelectrochemistry[9] and microdisk array[10] have been utilized in order to improve the performance of diamond electrode in stripping voltammetry. Here, we demonstrate that the immobilization of bacteria on the diamond surface and the analytical application of this bacteria-diamond coupling electrode in stripping voltammetry. *Acidithiobacillus ferrooxidans* is used in this work as its ability to tolerate high metal concentration and fix metal ions[11].

2. Experimental

2.1. Diamond Electrode Preparation

The submicron grain size, 5 μm thick microcrystalline diamond was grown on silicon (100) substrate at a substrate temperature of 750°C using hot filament chemical vapor deposition (conditions: 2 sccm CH_4, 200 H_2. 20 Torr for 7 h). Diamond samples were cleaned and chemically oxidized with hot 'Piranha' solution

(30% H_2O_2 : 97% H_2SO_4 = 1 : 3) for 1 hour, followed by rinsing with ultrapure water. The samples were then rinsed with tetrahydrofuran followed by hexane. Cleaned diamond samples were hydrogen-terminated by hydrogen plasma treatment at 800 W in a microwave plasma CVD system.

2.2. Bacteria-modified Diamond Electrode

In order to eliminate the effect of iron in the electrochemical measurement, iron-free cell suspension was prepared for the bacteria adsorption. The culture was first centrifuged at 1500g to remove the iron precipitates and the bacterial cells in the supernatant were harvested by centrifugation at 12000g. Cells pellet was washed 3 times with dilute H_2SO_4 and finally suspended in the acidic sterilized water. (pH 2). The adsorption of bacteria was carried out by immersing the diamond electrode into an iron-free bacterial suspension for a period of 6 hours.

2.3. Stripping Voltammetry

All electrochemical measurements were carried out in a single-compartment Teflon cell with a three-electrode configuration system: a diamond working electrode, Ag/AgCl reference electrode (3.0 M KCl), and a Pt wire counter electrode. For all electrochemical experiments, a small area (0.07 cm^2) of the diamond surface was exposed to the solution through a Viton O-ring. The top contact with the diamond sample surface was made through an Au-plated probe. The bacteria-modified diamond electrode was immersed in the Pb^{2+} working solution for a period of 6 mins before the stripping voltammetry was started. The cathodic stripping voltammetry was performed in the following parameters: deposition potential 1.65 V, deposition time 60 sec, scan rate 25 mV s^{-1}. Pb^{2+} working solution was prepared in 0.1 M HNO_3 solution.

3. Results and Discussions

3.1. Adsorption of Acidithiobacillus ferrooxidans

Micron-sized *Acidithiobacillus ferrooxidans* had developed a compact biofilm on top of the diamond electrode after immersing in the iron-free bacteria suspension for 6 hours. According to ZoBell's model of bacterial adhesion, the immobilization process can be divided into 2 stages—an initial stage of reversible adhesion followed by time-independent irreversible stage.[12,13]. Adhesion of bacteria onto the substrate is mainly attributed to the production of extracellular polysaccharides (EPS) which overcome the electrostatic barrier for cell adhesion. In another study by Gehrke *et. al.*, the author discovered that the EPS is in the deficient state in the iron-rich suspension[14]. Hence, iron-free bacteria suspension was used in this study for the adsorption process in order to induce large quantities of EPS for cell attachment. Furthermore, the presence of iron in the suspension might introduce interferences to the electrochemical measurement.

3.2. Linear Range and Detection Limit.

Cathodic stripping voltammetry is used in this work for the Pb^{2+} ions detection. The Pb^{2+} ions are first oxidized and deposited onto the electrode surface as PbO_2. At the cathodic scan, they are reduced back to Pb^{2+} ions form. Figure 1 shows the linear sweep voltammograms obtained from bacteria-modified diamond electrode in a series of Pb^{2+} concentration working solutions after initial contact period and deposition period. It can be clearly seen that the voltammetry plots exhibit a well-defined stripping peak at ~1.21 V. This stripping peak corresponds to the reduction of surface bound lead oxides back to the lead ions.

A linear dependence of the stripping current as a function of Pb^{2+} concentration can be established in the range of 10 μM to 100 μM for both bacteria-modified diamond electrode and bare diamond electrode (Figure 2). At higher Pb^{2+} concentration (>200 μM), the relationship with the stripping current becomes non-linear because not all the electrodeposited lead oxides can be reduced back within the stripping region[15]. It is also interesting to note that bacteria-modified diamond electrode exhibits higher sensitivity (37.5 nA μM^{-1}) towards Pb^{2+} compared to bare diamond electrode (22.3 nA μM^{-1}). The detection limit for bacteria-modified diamond electrode is found to be 10 μM, 2 times better than the detection limit for bare diamond electrode (20 μM). An explanation for the enhancement in detection limit and sensitivity is the ability of *Acidithiobacillus ferrooxidans* to fix metal ions, resulting in an increase of metal ions around the bacteria cells. This will provide the preconcentration effect which will directly enhance the detection limit.

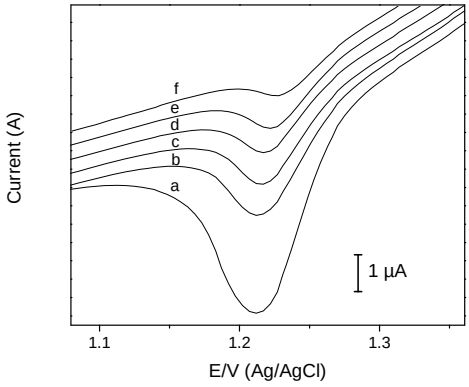

Fig. 1: Effect of lead concentration (a)100 µM, (b)50 µM, (c)40 µM, (d)30 µM, (e)20 µM, (f)10 µM. on cathodic stripping scans.

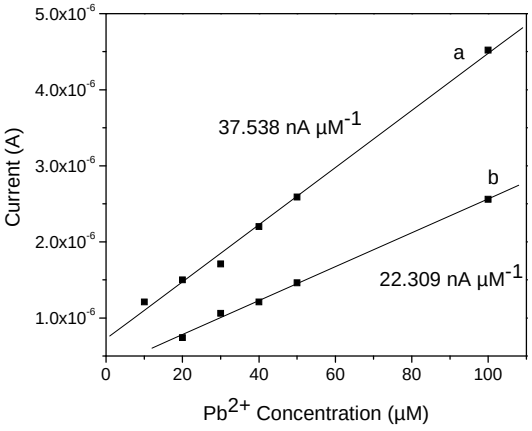

Fig. 2: Calibration plot for stripping current vs. different lead concentration for (a) bacteria-modified diamond electrode and (b) diamond electrode.

3.3. Adsorption Period

In order to achieve optimum bacteria adsorption**,** the adsorption period of the bacteria onto diamond electrode was investigated and shown in Figure 3. The optimum adsorption time was found to be 6 hours immersion of diamond electrode in the bacteria suspension. There is a linear increase in the stripping current for the adsorption period below 6 hours as the bacteria cells continuously attach to the diamond surface, further increase the local concentration of Pb^{2+} around the electrode during the stripping voltammetry process. However, the stripping current drops if the adsorption period over 6 hours. This may be attributed to the fact that the bacteria cells form thick colonization on the surface of electrode and this thick biofilm will hinder the electron transfer during the deposition process.

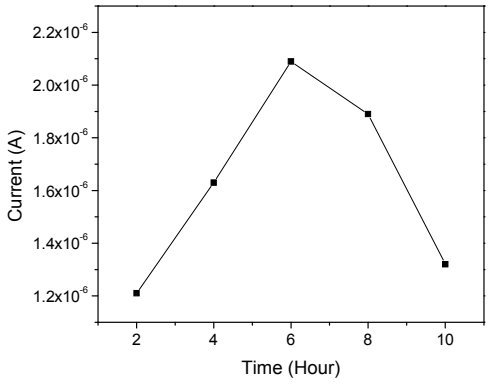

Fig. 3: Dependence of stripping currents on bacteria adsorption time onto diamond electrode.

3.4. Interference with Copper Ion

The effect of possible interferences from other metal ions towards Pb^{2+} detection was also investigated on the bacteria-modified diamond electrode. Linear sweep voltammograms in Figure 4 shows the effect of different copper concentration (ranging from 200 µM to 25 µM) on Pb^{2+} (constant concentration at 100 µM) stripping peaks. There are two stripping peaks which can be assigned to the reduction of lead oxides (ca. 1.21 V) and copper oxides (ca. -0.3 V). Linear sweep voltammograms in Figure 5 show the effect of copper (constant concentration at 500 µM) on different concentration of Pb^{2+} (ranging from 80 µM to 30 µM). It should be noted that the linear relationship of Pb^{2+} stripping currents is not affected by the presence of copper ions. No suppression of lead stripping peak is observed though the concentration of copper is found to be 15 times higher than the Pb^{2+} concentration. It is reported on glassy carbon electrode that the presence of 10-times excess of copper ions will completely suppress the lead stripping peak[16].

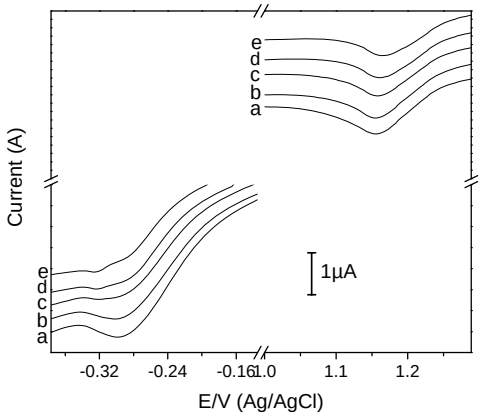

Fig. 4: Effect of different copper concentration on the stripping voltammograms recorded in constant concentration of Pb^{2+} working solutions (100 µM). Different copper concentrations (µM) (a) 200, (b) 150, (c) 100, (d) 50, (e) 25.

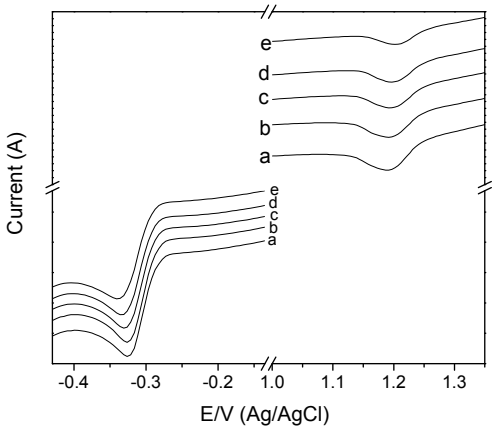

Fig. 5: Effect of constant copper concentration (500 µM) on stripping voltammograms recorded in different concentrations of Pb^{2+} working solutions. Different Pb^{2+} concentrations (µM) (a) 100, (b) 80, (c) 60, (d) 40, (e) 20.

4. Conclusions

This work demonstrates that the adsorption of bacteria cells, namely *Acidthiobacillus ferrooxidans* onto the diamond electrode can enhance the performance of the latter in stripping voltammetry. In lead ions detection, the bacteria-modified diamond electrode exhibits lower detection limit (10 µM) and higher sensitivity compared to bare diamond electrode. Under similar experimental parameters, this detection limit (10 µM) is comparable to the reported detection limit (3 µM) by ultrasonically-assisted stripping voltammetry with diamond electrode[17]. The capacity of *Acidthiobacillus ferrooxidans* to fix heavy metal ions on the membrane enables it to act as the preconcentration agent to increase the local concentration of heavy

metal ions around the electrode. The bacteria-modified diamond electrode is also found to be free from the interference of intermetallic species.

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6. References

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