Screen-printed electrochemical biosensors based on magnetic core-shell nanoparticles

Longinotti, G.; Lloret, P.; Ybarra, G.; Moina, C.
Centro de Procesos Superficiales, Instituto Nacional de Tecnología Industrial (INTI), Buenos Aires, Argentina.
Hermida, L.
Centro de Química, Instituto Nacional de Tecnología Industrial (INTI), Buenos Aires, Argentina.
Milano, O.; Roberti, M.; Malatto, L; Fraigi, L.
Centro de Electrónica e Informática, Instituto Nacional de Tecnología Industrial (INTI), Buenos Aires, Argentina.
moina@inti.gov.ar

Abstract. In this work, the design, manufacture and characterization of biosensors based on thick film (screen printing) technology is presented for the electrochemical determination of the catalytic activity of enzymes immobilized onto superparamagnetic iron oxide nanoparticles (SPION).

Keywords: *Biosensors, Photolithography; Screen Printing; SPION; Enzymes.*

I. INTRODUCTION

Enzymatic electrodes are a type of biosensors formed by an electrode on whose surface a redox enzyme has been immobilized [1]. Enzymes catalyze reactions producing redox substances which can be electrochemically detected at the electrode; thus, the resulting current is a measure of the reaction rate. Usually, the analytes in enzymatic electrodes are the substrates of the enzymes (i.e. the substance whose reactions are catalyzed by the enzyme); in this case, a higher current at the electrode is a consequence of a higher concentration of the analyte. Enzyme inhibitors (i.e. substances which inhibit the catalytic activity of the enzymes) can also be regarded as analytes. For instance, acetylcholinesterase (AChE) is an enzyme which participates in neurotransmission process and is inhibited by organophosphorus and carbamate insecticides. In these cases, a lower current at the electrode is a consequence of a higher concentration of the analyte.

The immobilization of enzymes onto nanoparticles and the subsequent attachment of the nanoparticles onto an electrode is an attractive alternative in this context; especially in the case of magnetic nanoparticles which can be attracted to and removed from the electrode by the action of magnetic fields. Such enzyme modified magnetic nanoparticles may be used in a pre-concentration step for the determination of inhibitory substances. Another attractive aspect is the possibility of using a single working electrode for the determination of multiple, sequential analytes simply by changing the enzymes attached to the nanoparticles.

In this work, the feasibility of the electrochemical determination of the catalytic activity of horseradish peroxidase (HRP) immobilized onto chitosan modified superparamagnetic iron oxide nanoparticles (SPION) employing a miniature electrochemical cell is presented.

II. EXPERIMENTAL DETAILS

Electrodes design and manufacture

The sensor design, made up of three electrodes, was developed on the basis of a previous one carried on Au electrodes deposited on Si and glass substrates. Thick film electrodes were printed by conventional screen printing technology. Commercial Au organometallic paste (ESL D8083) and 96 % α-Al_2O_3 substrates were employed. The three-electrode layout was transferred by means of photolithography to a stainless steel mesh (400 wires per inch) with a photosensitive film (Ulano CDF-2).

Enzymatic electrodes

HRP was immobilized on screen printed Au electrodes employing cysteine and glutaraldehyde as molecular linkers between the gold electrode and the enzyme.

Nanoparticles synthesis and enzyme immobilization

Chitosan coated SPION were obtained by a co-precipitation method [2]. Briefly, a solution containing 0.5 % chitosan and stoichiometric quantities of Fe^{2+} and Fe^{3+} was mixed with NH_4OH solution under stirring. After 30 min, the precipitate was magnetically collected, washed, re-dispersed in water, and stored. The quantity of chitosan in the shell, determined by the ninhydrin method [3], was 12 mg per 100 mg of SPION.

Chitosan coated SPION were immersed in a 7 % glutaraldehyde solution overnight, washed with deionized water, immersed in 0.1 M phosphate buffer of pH 7 containing a 2.49 U μl^{-1} HRP during 2 hours, and finally washed six times with 100 µl of deionized water.

The catalytic activity of the HRP on the SPION was confirmed with a colorimetric technique. When 4-aminoantipyrine is employed as an electron donor by HRP during the oxidation of H_2O_2, it generates a colored substance (quinoneimine) in the presence of phenol. 50 µl of an aqueous dispersion of SPION was added to 3 ml of 0.1 M phosphate buffer of pH 7 containing 0.4 mM 4-aminoantipyrine, 0.63 mM phenol 0.63 and 0.8 mM H_2O_2; the absorbance at 500 mm was recorded.

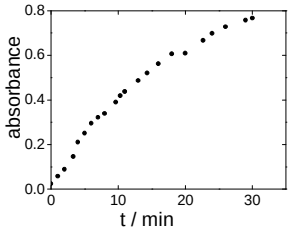

Fig. 1. Time evolution of the absorbance at 500 nm of SPION-HRP.

Electrochemical measurements

Current-potential curves were obtained at a scan rate of 20 mV s^{-1} in a 0.1 M phosphate buffer of pH 7 + 0.1 M KCl. 4 mM hydroquinone was employed as a redox mediator and the H_2O_2 concentration was increased from 0.2 to 1.2 mM. Ag | AgCl was employed as a reference electrode. SPION were collected with the aid of a magnet and concentrated on the surface of the screen printed gold electrode.

III. RESULTS

The time evolution of the absorbance of the SPION-HRP is shown in Fig. 1. The catalytic activity of the enzyme is retained after the immobilization onto the nanoparticles. Fig. 2 shows the current-potential curves obtained for the electrodes with enzymes immobilized onto Au electrodes (Fig. 2a) and enzymes immobilized onto SPION (Fig. 2b). The resulting calibration curves are presented in Fig. 3. The sensitivity is quite similar for both configurations.

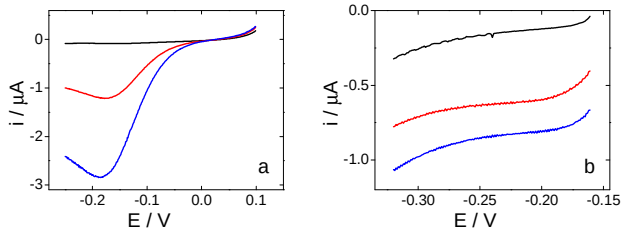

Fig. 2. Current-potential curves obtained with solution of *y* mM H_2O_2 (*y* = 0, 0.6, 1.0). a) Enzymes immobilized onto Au electrodes; b) Enzymes immobilized onto SPION.

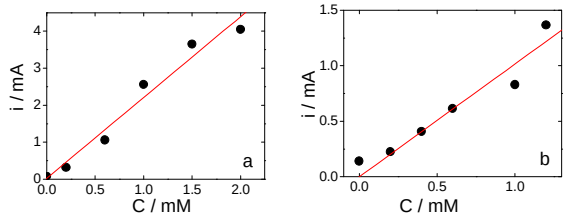

Fig. 3. Current (at -0.190 V) vs. H_2O_2 concentration curves obtained for: a) Enzymes immobilized onto Au electrodes; b) Enzymes immobilized onto SPION.

IV. CONCLUSIONS

The electrochemical response of the enzymes immobilized onto SPION was comparable to that obtained for enzymatic electrodes. Measurements in a conventional electrochemical macro-cell were performed in order to asses the influence of the scaling-down in the response of the devices.

These results open the possibility for the design and fabrication of biosensors for monitoring the quality of waters employing enzymes immobilized onto SPION as preconcentrators.

V. REFERENCES

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