Development of a bifunctional Pt/Au platform microelectrode modified with glucose oxidase for glucose determination

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Glucose is a reducing sugar very important in the human diet and is abundant in many foods, such as fruit juices, honey, and yogurt and can be easily ingested and metabolized. It provides the energy for the human body perform its healthy functioning. However, many metabolic imbalances associated with variations in the level of glucose in the blood, urine or saliva [1]. Therefore it is necessary to develop methods cheap, simple and quick to allow the monitoring of this metabolite.

The development and characterization of highly catalytic surface to be used as electrochemical platforms is an important area in biosensor research. However, despite such interest, aiming to increase the analytical signal, there are relatively few reports in this subject. A much higher number of communications in biosensors area is related to modifications related to the enzymes such as different types of immobilizations or the use of any redox mediators [2].

In this sense, this work describes the development of a Pt microelectrode (UME-Pt) surface modified with electrodeposited Au nanostructures. To improve the biocompatibility and the electrochemical detectability of reactions products, the Au nanostructures were covered with a cystamine (Cys) self-assembled monolayer for enzyme immobilization while the UME-Pt surface was also modified with ferrocene (Fc) to enhance the product detection.

For detection of glucose with lower detection limits and a further application in saliva constructed a Pt microelectrode (UME-Pt) of 50 mm in diameter embedded in the glass capillary while keeping the tip of the microwire Pt exposed. For sealing the capillary was filled with epoxy resin. After the curing time of the resin, the electrode was polished. A resin finishing Araldite ® was made at the other end of the glass tube to avoid breaking the electrical contact during use [3].

The deposition of gold nanostructures was obtained by linear sweep voltammetry from 1.4 to -0.2 V *vs.* Ag/AgCl/KCl 3 mol L^{-1} at 0.010 V s^{-1} in a solution of $HAuCl_4$ $1x10^{-3}$ mol L^{-1} in H_2SO_4 0.1 mol L^{-1}. The nanostructures were analyzed by atomic force microscopy and voltammetric experiments with $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$. The Au surface was further modified by depositing a cystamine through immersion by 6 h after that the UME-Pt surface was also modified by 10 µL aliquot the ferrocene 0.01 mol L^{-1} in methanol.

In the next step, 10 µL of a glucose oxidase (GOx) stock solution (containing 1000U) was dropped on the electrode surface followed by 5 µL of 0.5% glutaraldehyde and the electrode was allowed to dry at room temperature. The electrode was then immersed in pH 7 phosphate buffer solution for 15 min and washed. Figure 1 shows a schematic model bifunctional surface.

The oxygen peroxide generated in the enzymatic reaction was followed by cyclic voltammetry, to check the voltammetric profile and chronoamperometry in order to construct the calibration curve, applying 0.25 V to the electrode, for 60 s.

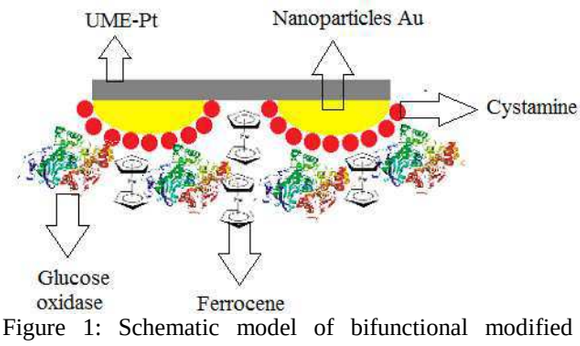

Figure 1: Schematic model of bifunctional modified microelectrode surface.

The bifunctional Pt/Au/Cys/GOx/Fc electrode was able to oxidized the enzymatically generated oxygen peroxide in 0,25 V. The reactions are described below:

$$Glicose(FADH_2) + O_2 \rightarrow Glicose(FAD) + H_2O_2$$
$$Fe^{2+} \rightarrow Fe^{3+} + e^-$$
$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$
$$2Fe^{3+} + H_2O_2 \rightarrow 2Fe^{2+} + O_2 + 2H^+$$

In order to test the applicability of the developed sensor in analytical work, a calibration curve was constructed. The linear range for glucose detection was from 0.4 to 2.9 µmol L^{-1}. The linear relationship between diffusion currents and analyte concentration was represented by I_{pa} (A) = 5.03 x 10^{-11} (A/mol L^{-1}) + 2.01 x 10^{-4} [glucose] (mol L^{-1}), with a correlation coefficient of 0.987 (for n = 7). The detection limit was estimated as 30 nmol L^{-1} by the IUPAC procedure.

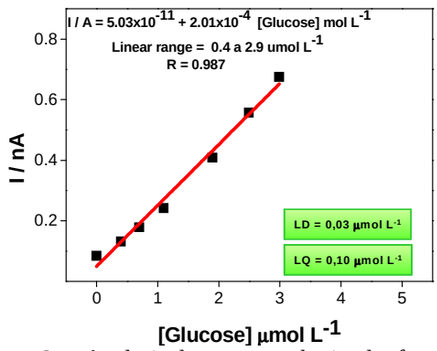

Figure 2: Analytical curve obtained for glucose employing the bifunctional UME electrode modified with Pt/Au/Cys/GOx/Fc in PBS (pH = 7) with constant potential of 0.25 V *vs.* Ag/AgCl.

The low value in the detection limit suggests that the Pt/Au/Cys/GOx/Fc biosensor is suitable for the detection of glucose in matrices of interest.

References

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