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# NANOSTRUCTURED GOD/$TiO_2$ /SCPE ELECTRODE FOR AMPEROMETRIC GLUCOSE BIOSENSORS

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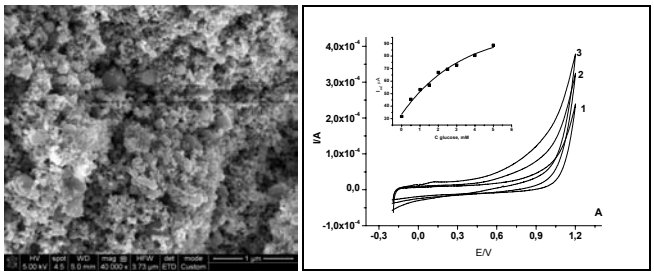
A porous $TiO_2$ material is further explored for protein immobilization and biosensing. We presented a simple and effective method to immobilize the glucose oxidase in $TiO_2$ matrix. The screen-printed carbon electrodes were used for support for biosensors. The GOD/$TiO_2$/SCPEs modified electrodes were characterized by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and FTIR spectroscopy. Amperometric detection of glucose with The GOD/$TiO_2$/SCPE sensor at 0.7 V (vs.ag/AgCl) resulted in a linear response in the concentration range 0-2.5 mM.




## INTRODUCTION

Since the 1990's, the interest in enzyme electrochemistry has been focused on incorporating enzyme into various films modified on electrode surface.[1,2] The film phase is different from the solution phase, and may provide a better and more suitable microenvironment for the enzyme to directly exchange electrons with underlying electrodes. Nanomaterials, with their unique and excellent properties such as large surface area and good biocompatibility, have been utilized as the film forming materials to immobilized GOD on elecrodes.[3] Porous $TiO_2$ may be a promising material for enzyme immobilization owing to its high biocompatibility and large specific surface area of nanosized particles.[4] In the last ten years the research of the synthesis of nanosized porous $TiO_2$ materials and its applications in electro analytical chemistry has been rather intense.[5-7]

The $TiO_2$ nanoparticles are able to exhibit some special properties in structure and electrochemical performance. On the other hand, besides its advantages of optical transparence, large surface area and its preference of selective interaction with some groups,[8] it has been demonstrated that the porous $TiO_2$ material exhibit high ability in protein loading.

In this work we propose to immobilize the glucose oxidase on a nanostructured $TiO_2$ matrix for the development of an amperometric glucose biosensor.

## RESULTS AND DISCUSSION

Methods for the fabrication of GOD/$TiO_2$/ SCPE electrodes are presented in experimental part. As shown, GOD/$TiO_2$/SCPE electrodes are obtained in two steps: (1) the immobilization of GOD onto $TiO_2$ matrix; (2) the resulting solution was casting onto the surface of SCPE electrode.

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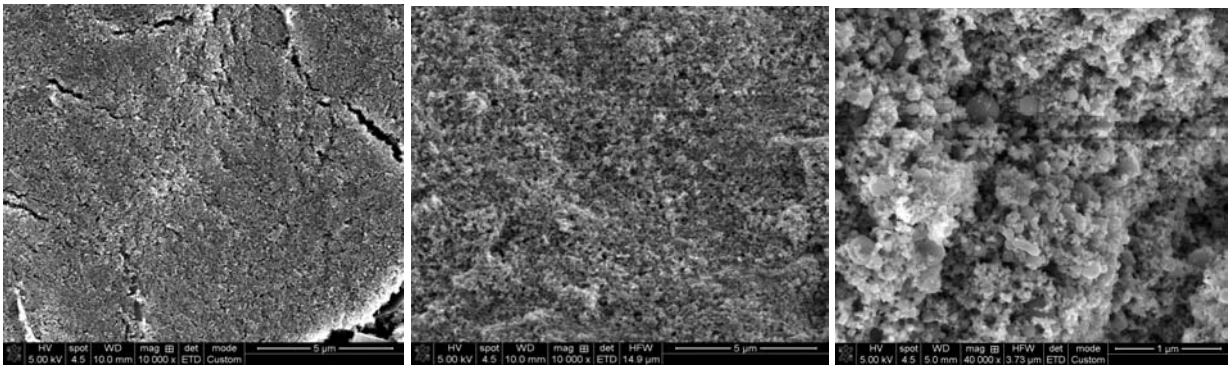


(a) (b) (c)

Fig. 1 – SEM images of: (a) SPCE; (b) and (c) GOD/$TiO_2$/SCPE (large scale 1-5 μm).

The immobilisation of glucose oxidase on $TiO_2$ nanoparticles revealed that the white color change of the $TiO_2$ nanopowder from white to yellow. In order to compare the electrochemical performance of GOD/$TiO_2$/SCPE electrode a SCPE electrode was modified with $TiO_2$ nanoparticles in the same conditions. The structure and morphology of all prepared electrodes were examined by scanning electron microscopy (SEM) and atomic force microscopy (AFM), and the effectiveness of enzyme immobilization was further examined by FTIR spectroscopy.

SEM pictures revealed visual difference of the surface of the electrodes before and after modification with GOD immobilize on $TiO_2$ nanoparticles. It can be seen the porous structure of the GOD/$TiO_2$/SCPE surface, indicating that the $TiO_2$ matrix is higly porous in nature. The porous structure of the $TiO_2$ matrix provided significantly enhanced effective electrode surface for enzyme immobilization. When the GOD was entrapped, it was obvious that the porous structure was retained and the aggregates of the trapped enzymes were observed. The porous structure of matrix and the large specific surface area of $TiO_2$ nanoparticles make this material suitable for the development of biosensors.

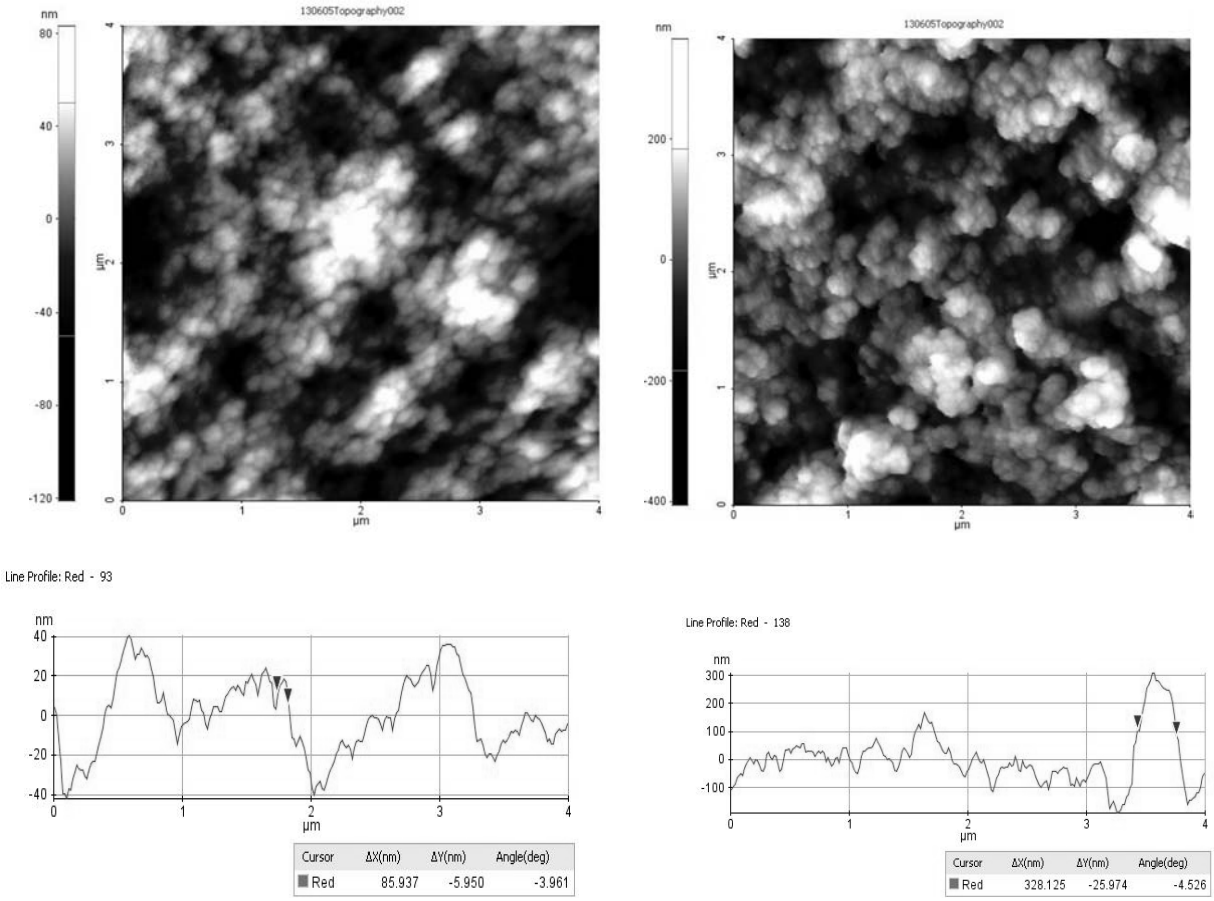
The surface became rough after enzyme immobilization on $TiO_2$ matrix. The roughness of the electrode surface was analyzed by AFM. In Fig. 2 are presented the AFM images of the $TiO_2$ and GOD/$TiO_2$ modified SCPE electrodes. The AFM parameters have been evaluated for 20x20 $\mu m^2$ surface area. It is significant that there are morphological differences between the electrodes. Thickness of the titanium dioxide substrate was found to be 40 nm and 200-300 nm by immobilization of GOD. The height difference between the bright region and the dark ground was about 20 nm for $TiO_2$/SCPE surface electrode and 100 nm for GOD/$TiO_2$/SCPE surface, respectively. The spherical shaped structures of GOD in the film indicated that GOD was immobilized on the $TiO_2$ nanoparticles.

FTIR spectroscopy, which could provide some useful information on the structure and conformation of enzyme molecules, was employed to investigate the existing state of the immobilized enzyme. For native GOD, the adsorption band in the range from 1800 $cm^{-1}$ to 1000 $cm^{-1}$ is assigned to the characteristic peaks of stretching vibration of carbonyl groups, bending vibration of amide groups, methyl and methylene groups, and sugar rings in GOD.[9]

Comparison of the FTIR spectra of GOD/$TiO_2$/SCPE with that of $TiO_2$/SCPE revealed a large difference of the transmittance in the range from 1800$cm^{-1}$ to 1000 $cm^{-1}$. For $TiO_2$/SCPE sample two peaks appeared at 1407 $cm^{-1}$ and 1170 $cm^{-1}$, respectively, while for GOD/$TiO_2$/SCPE sample another two peaks appear at 1640 $cm^{-1}$ and 1080 $cm^{-1}$ which are in a good agreement with the peaks for native GOD, indicating the functional groups in enzyme.

Before electrochemical measurements, all the prepared electrodes were kept in 0.05M PBS (pH=6.5) solution for 30 minutes for removing the residuals.

Cyclic voltammetry was used to study the electrochemical behavior of the SCPEs modified with $TiO_2$ ($TiO_2$/SCPE) and GOD (GOD/$TiO_2$/SCPE) in PBS (pH 6.5) containing 5mM $[Fe(CN)_6]^{3-/4-}$ redox probe (Fig.4), which was usually utilized to characterize the surface feature of the electrode. The high values of current for both electrodes can be attributed to a higher active surface area of $TiO_2$. As shown, the anodic and the cathodic peaks were quite close but the ΔEp were relatively large (122 mV for GOD/$TiO_2$/SCPE and 220 mV for $TiO_2$/SCPE) suggesting a sluggish electron transfer kinetic.




**(a)** **(b)**

Fig. 2 – AFM images and section analysis of: (a), $TiO_2$/SCPE and (b), GOD/$TiO_2$/SCPE electrodes.

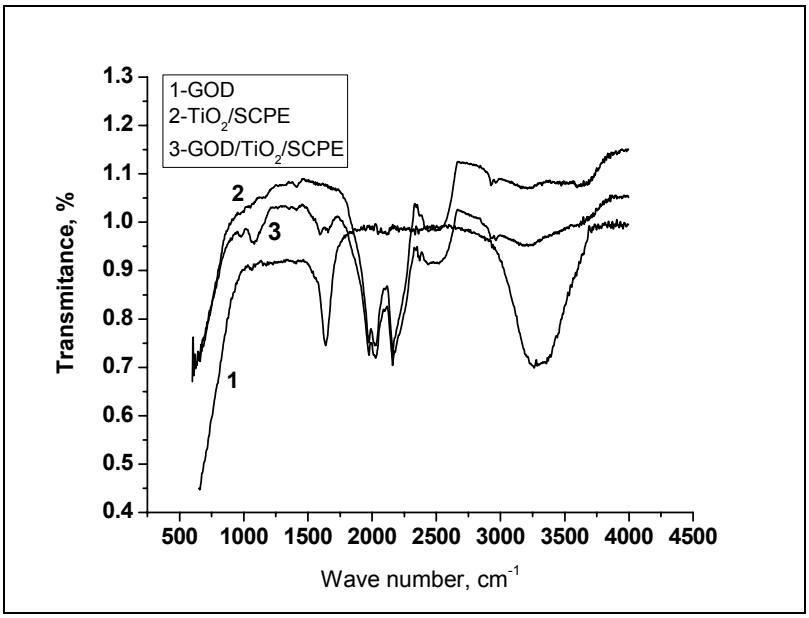



Fig. 3 – FTIR spectra of: (1), pure GOD; (2), $TiO_2$/SCPE and (3), GOD/$TiO_2$/SCPE electrodes.

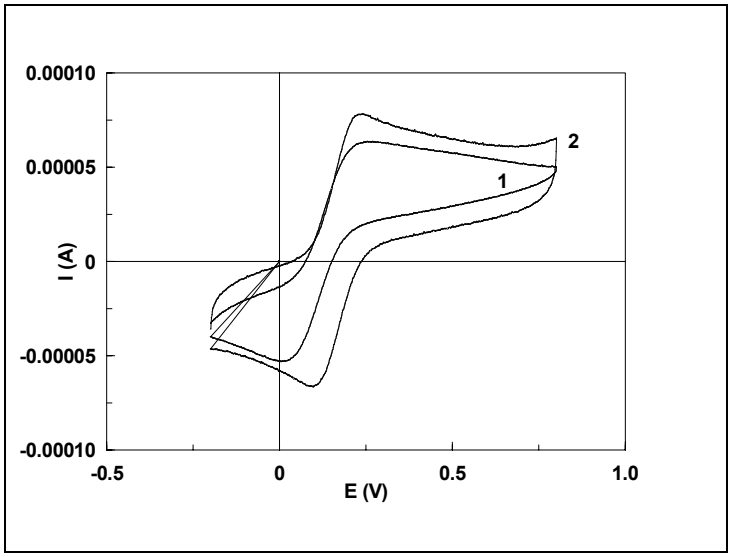



Fig. 4 – Cyclic voltammogrames recorded on $TiO_2$/SCPE (1) and GOD/$TiO_2$/SCPE (2) electrodes in 0.05M phosphate buffer solution +5mM $[Fe(CN)6]^{3-/4-}$; pH 6.5; scan rate: 50 $mVs^{-1}$.

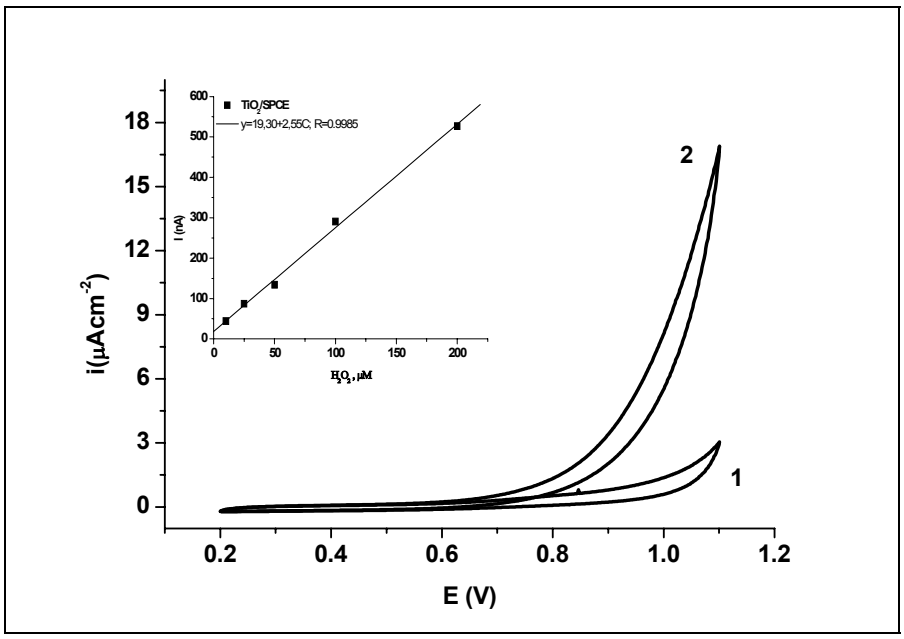



Fig. 5 – Cyclic voltammogrames recorded on $TiO_2$ /SPCE in 0.05 M phosfate buffer solution pH=6.5. Scan rate v = 50 $mVs^{-1}$. (1) buffer phosfate; (2) buffer phosfate + $H_2O_2$ 1mM. In set: Calibration plot for detection of $H_2O_2$, using chronoamperometry on $TiO_2$/SCPE electrode; applied potential E = 0.7V.

Fig. 5 shows the electrocatalytic properties of the $TiO_2$/SCPE electrodes to electro-oxidation of $H_2O_2$. It can be seen in Fig. 5 a large increase of the anodic current in the presence of $H_2O_2$ (curve 2). The catalytic current of hydrogen peroxide oxidation started at 0.6V vs. Ag/AgCl and a remarkable increase can be seen above 0.8V. The large surface area of SCPE modified with $TiO_2$ nanoparticles renders high electrocatalytic activity for $H_2O_2$ oxidation.

The calibration curve was obtained from amperometric measurements performed at 0.7 V applied potential for successive additions of $H_2O_2$ in 0.5 M PBS solution at pH=6.5 (Fig. 5 in set).

In order to assess the bioactivity of the immobilized GOD, the electrochemical response of the GOD/$TiO_2$/SCPE electrode was investigated as a function of glucose concentration.

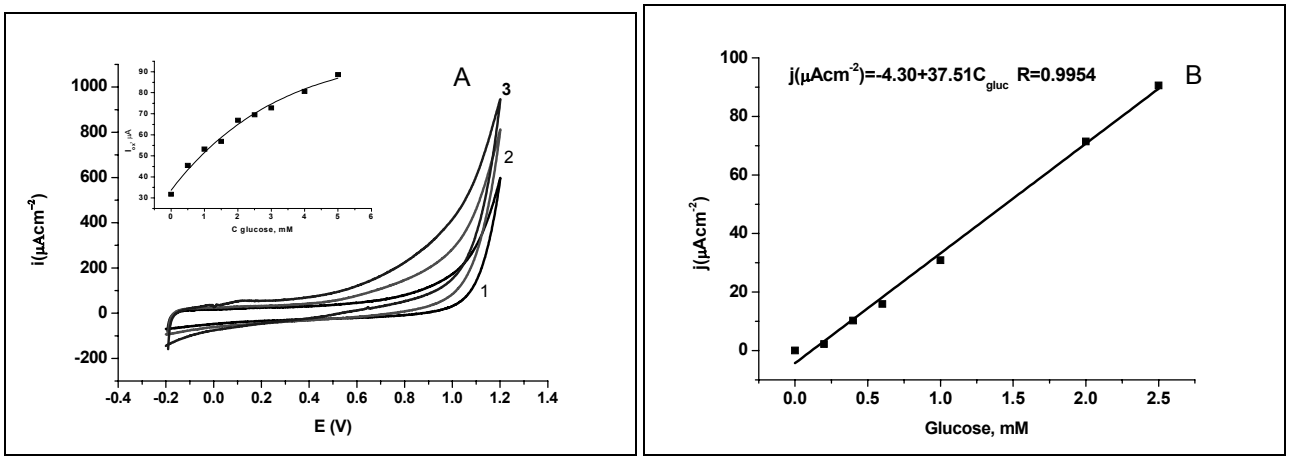



Fig. 6 – A. Cyclic voltammograms recorded on GOD/$TiO_2$ /SPCE in 0.05M phosfate buffer solution, pH=6.5. Scan rate v = 50 $mVs^{-1}$. (1) phosfate buffer; (2) phosfate buffer + glucose 1mM; (3) phosfate buffer + glucose 3mM. In set: Variation of catalytic current with glucose concentration. B. Calibration plot for detection of glucose, using chronoamperometry on GOD/$TiO_2$/SCPE electrode, Applied potential E = 0.7; V in phosphate buffer pH=6.5.

It is well known that GOD usually exhibits a good electrocatalytic activity toward oxygen and $H_2O_2$ when immobilized on electrode surface[10] and that the electrocatalytic reaction of GOD with glucose is a first–order reaction.[11]

The enzymatic reaction based on glucose oxidase is the following:

$$\text{Glucose} + O_2 \rightarrow \text{gluconolactone} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

The quantification of glucose can be achieved via electrochemical detection of $H_2O_2$. The hydrogen peroxide is detected at the $TiO_2$ nanoparticles.

The addition of glucose in buffer solution results in an increase of the oxidation current (Fig. 6, curves 2 and 3). Fig. 6A present the response current of GOD/$TiO_2$/SCPE electrode as a function of glucose concentration. The catalytic current increases with the increase of glucose concentration in the range from 0.01 mM to 5 mM. The repeatability of the GOD/$TiO_2$/SCPE electrode to glucose has also been characterized for six times by adding 0.25 mM glucose, which result in rather similar current responses with a relative standard deviation of 7.2%. The resulting calibration plot for glucose over the concentration range 0 mM to 2.5 mM is presented in fig. 6B. The linear response can be fitted with $i(\mu Acm^{-2})=-4.30+37.51C_{gluc}$ (mM) (with a regression coefficient of 0.9954) and thus the sensitivity of the electrode is 37.51 $\mu Acm^{-2}$ $mM^{-1}$ which is higher than sol-gel $TiO_2$ film/copolymer/GOD electrode (2.06 $\mu Acm^{-2}$ $mM^{-1}$)[12] or the Chit/GOD/$TiO_2$ nanofiber/Pt electrode (9.25 $\mu Acm^{-2}$ $mM^{-1}$)[13].

Based on these results we can say that GOD/$TiO_2$/SCPE electrode will have a promising application in amperometric detection of glucose.

## EXPERIMENTAL

### Reagents

All chemicals from commercial sources were of analytical grade. $TiO_2$ (titanium dioxide) 99% nanoparticles and glucose oxidase from Aspergillus niger, 6500 U/mL. Nafion (perfluorosulfonated ion-exchange resin, 5% (w/v) solution in a solution of 90% aliphatic alcohol and 10% water mixture were purchased from Sigma Aldrich. Sodium hydrogen phosphate $Na_2HPO_4 \cdot 2H_2O_2$, disodium hydrogen phosphate $KH_2PO_4$, KCl were purchased from Sigma-Aldrich. The electrolyte solution for electrochemical measurements and amperometric detection of glucose and hydrogen peroxide ($H_2O_2$) was phosphate buffer solution (PBS) with pH=6.5.

### Instrumentation

Electrochemical measurements were carried out using an Autolab potentiostat/galvanostat controlled by the GPES software and a PARSTAT 4000 potentiostat/ galvanostat controlled via VersaStudio software with FRA module. Screen-printed carbon electrodes (SPCE) model DRP-110 purchased from DropSens (Spain) were used for working electrodes. The screen-printed carbon electrodes (SPCE) for electrochemical measurements is composed by a graphite working electrode (d = 4 mm), a graphite auxiliary electrode and a silver pseudo-reference electrode, with silver electric contacts deposed on a ceramic substrate.

The morphology of the samples was investigated by scanning electron microscopy (SEM) using a high-resolution microscope, FEI Quanta 3D FEG model, at an accelerating voltage of 5 kV, in high vacuum mode with Everhart-Thornley secondary electron (SE) detector. Atomic Force Microscopy (AFM) measurement was carried in the non-contact mode with a XE-100 apparatus from Park System equipped with flexure guided, cross-talked eliminated scanners, using sharp

tips (< 7 nm tip radius; PPP-NCLR type from Nanosensors TM) of app. 225 μm length, 38 μm width and 48 N/m spring constant/ ~ 190 KHz resonance frequency. The topographical 2D AFM images were taken over the area of 20 * 20 $\mu m^2$ and used the horizontal by line flattening as planarization method for tilt (most probably due the thermal drift). Fourier-transform infrared spectroscopy (FTIR) was employed to investigate the existing state of the immobilized GOD. The measurements were carried with a spectrometer Brucker Optik Tensor 37.

**Preparation of electrodes**

Before surface modification the SCPEs were subjects of cleaning operation by applying a potential of 1.7V for 10 minutes.

GOD immobilization on $TiO_2$ nanoparticles was performed in two steps.

The first step was the immobilization of the glucose oxidase onto the $TiO_2$ nanoparticles: 200 mg of $TiO_2$ powder (nanoparticle 99%) was put into 5 ml of an aqueous GOD solution (5mg/mL), stirred for 30 minutes and stored at 4°C for a couple of days. Then, the incubated $TiO_2$ powder was filtered from the immobilization solution and rinsed with doubly distilled water thoroughly. The immobilization effectiveness could be verified by the color change of the $TiO_2$ powder from white to yellow and analyzed by FTIR.

The second step consists of the electrodes preparation. The GOD/$TiO_2$/SCPE electrode was prepared by depositing 5μL of the mixture which was made by blending of 1 mg of coated $TiO_2$ nanopowder with 10 μl of 0.5% Nafion solution, onto the electrode surface, and drying at 4°C for one day. The electrodes were stored in 0.05M phosphate buffer at 4°C in a refrigerator.

The $TiO_2$/SCPEs were achieved as the GOD/TiO2/SCPEs, a mixture of 1 mg of TiO2 nanopowder with 10 μL of 0.5% Nafion solution was cast onto the electrode surface and drying at the room temperature for 10 minutes.

## CONCLUSIONS

This work has developed a simple and effective method to fabricate a nanostructured GOD/$TiO_2$ material for biosensors. The experimental results show that the $TiO_2$ nanoparticles are also another kind of effective substrate for the immobilization of enzyme molecules.

Owing to its high biocompatibility, high adsorption, and little harm to the biological activity of enzyme molecules, $TiO_2$ nanoparticles have potential application values in the field of bioelectrochemistry and biosensors.

The improvement of the amperometric response of the GOD/$TiO_2$/SCPE electrode can be attributed to its excellent electrocatalytic activity to $H_2O_2$ electrooxidation and effective GOD immobilization on the electrode surface. Further research for optimize the experimental variables for biosensors application of this nanostructured material in our laboratory is currently ongoing.

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