OPTICAL PROPERTIES OF GLUCOSE BIOSENSORS BASED ON ITO-DENDRIMER NANO-INTERFACES

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ABSTRACT

In this work the properties of a thin film of indium tin oxide (ITO) contact as a hole-injecting material for the design of glucose biosensors based on the bioconjugate poly(amido amine) dendrimers (PAMAM)-Glucose oxidase (GOx) thin films was investigated. The optical properties of the ITO films changed significantly by increasing substrate temperature and reaches to a maximum transmission of 90% for ITO film deposited at 500 °C. It was observed an improved transmission coefficient that could be due to the ITO structure coupled with reduction in the number of charge carriers. Results of XRD analysis revealed that the ITO films become polycrystalline when deposited at higher substrate temperature (400°C). A conductivity of 5,6.10^{-4} (Ω.m) has been obtained with thickness of 100 nm and annealing of 550°C in the nitrogen atmosphere. The obtained PAMAM-GOx-ITO biosensor exhibited good stability, reproducibility and high sensitivity for the determination of glucose.

Keywords: glucose biosensors, ITO, PAMAM, dendrimers.

1. INTRODUCTION

In the last decades indium tin oxide (ITO) films has attracted great attention due to their optoelectronic properties. Indium tin oxide (ITO) is a degenerated semiconductor with lower gap, formed by a mixture of indium (III) oxide (In_2O_3) and tin (IV) oxide (SnO_2), typically 90% In_2O_3, 10% SnO_2 by weight.

Several techniques have been reported for producing ITO thin films, including sputtering, chemical vapor deposition (CVD), laser ablation and sol-gel process. The CVD method require relative low temperature, low cost, ease handle and has the advantage of relative ease for large scale application, whitch is important for disponsable electrodes.

Recently, vigorous research activity in the area of polymers with controlled architectures has been observed. [1-4] Actually, a high level of structural control can be attained in the synthesis of highly branched macromolecules denominated dendrimers.

Dendrimers display a treelike isomolecular architecture having highly branched chains and many exterior end groups, whitch can mimicry the properties of globular proteins and regular micelles, what make the dendrimers an interesting macromolecule for the design of biosensors.

Today, polyamidoamine (PAMAM) dendrimers are the more widely known dendrimer well characterized and commercialized, being the first complete dendrimer family synthesized, well characterized and commercialized. Due to raised density of functional NH_2 peripheric groups in the periphery of the macromolecule, PAMAM seems to be an ideal system for the project contends oxi-reducing enzymes. Previous works showed also the aplication of dendrimers support for developed of amperometrics biosensors. [5]

In this work we investigate the properties of a thin film of ITO contact as a hole-injecting material for the design of glucose biosensors based on the bioconjugate PAMAM-GOx. The fairly large electron affinities (or work function) of ITO thin films may be adequate to improve the electron transfer processes at redox-enzyme interface of the PAMAM-GOx nanobiosensor.

2. EXPERIMENTAL

2.1. DENDRIMER SYNTHESIS AND CHARACTERIZATION

PAMAM dendrimers was synthesized by divergent route using as precursor a monomer type AB2 resultant of the Michael condensation between methyl acrylate and 4-amino-benzoic acid. The polymerization reaction was conduced at 160 ºC for 30h in argon atmosphere. The obtained PAMAM dendrimer was purified by dialysis, getting a dendrimer with $\overline{M}_n = 2.6\,\mathrm{kDa}$ (GPC), polydispersion index 1,15 and generation 4 (G4). In order to investigate the molecular structure of PAMAM and the eletronic transport, FTIR spectroscopy (Perkin Elmer S2000), molecular simulation using PM3 semi-empirical calculations (Hyperchem 7.0) and the IxV curve (electrometer Keithley 237) were used.

2.2. ITO SYNTHESIS AND CHARACTERIZATION

ITO films were deposited by spray system in glass substrate (Corning 7059) using an atomizer. A methanol solution of indium chloride ($InCl_3$. 3.5 H_2O, 97%) and tin chloride ($SnCl_4$, 97%) was used. The substrate temperature was fixed in 300 ºC and the heat treatment was 550 ºC during 1 hour. The optical properties of the obtained ITO thin film (band-gap or absorption coefficient) were determined by using UV/Vis spectroscopy on a Varian 634 spectrophotometer. The study of crystalline ITO phases were investigated by the X-ray diffraction technique (XDR) using a horizontal diffractometer D/MAX-200. The X-ray patterns were taken from monochromatized CuKα radiation source (λ = 1.5418 Å). The thickness of the films was determined by laser interferometry method.

2.3. PREPARATION OF THE BIOSENSOR ELECTRODES

A multienzymatic system was obtained after the immobilization of the GOx and peroxidase (HRP) on the dendrimer surface via Schiff base reaction.[4] Monolayers of the bioconjugate PAMAM-GOx-HRP was then immobilized onto ITO thin films.

2.4. ELECTROCHEMICAL MEASURENMENTS OF THE BIOSENSOR SIGNAL

The measurenments of biosensor signal was obtained by recording of peak currente at 100 mV (maximum current) produced in glucose oxidation at room tempetature by GOx. An electometer Keithley 237 connected with computer (Labview 7.0 software) was used to register the resultant current generated at the ITO interface.

3. RESULTS AND DISCUSSION

The optical properties of the ITO films changed significantly by increasing substrate temperature and reaches to a maximum transmission of 90% for ITO film deposited at 550 °C. The surface of the ITO thin film was smooth and presents high transparency (Figure 1) as the result of nanosize crystallites of ITO. It was observed an improved transmission coefficient that could be due to the ITO structure coupled with reduction in the number of charge carriers.

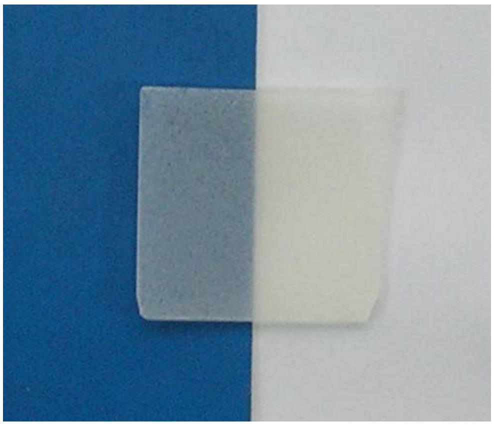


FIGURE 1: The synthesized ITO thin film on glass substrate.

The UV/Vis spectrum give an estimate value for the ITO film band gap. [(6)] The associated band gap energy (3.21 eV) to a maxima absorption band at 386 nm and (3.13 eV) at 366 nm for the In_2O_3 and ITO, respectively, is given by Figure 2, characterizing the property of the semiconductor structures. The low conduction for this kind of material is due to the oxygen fault in the oxide indium lattice creating acceptors states under the conduction band. In the ITO thin films, the substitution of the In^{+3} by Sn^{+4} could create more acceptors states.

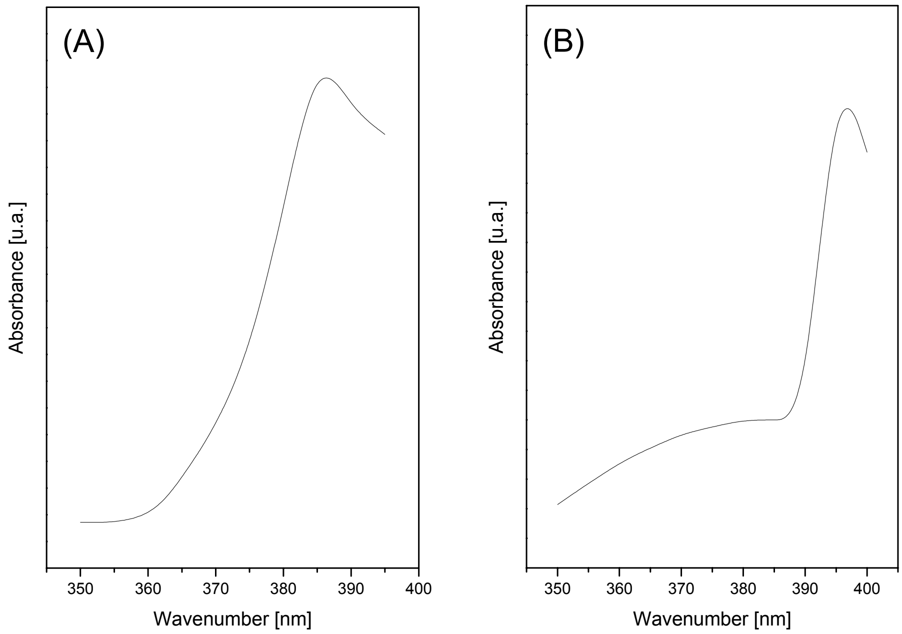


Figure 2: Absorption UV/Vis spectra for the indium oxide (A) and ITO (Sn 11.5 %) (B).

Results of the XRD analysis revealed that the ITO films become polycrystalline when deposited at higher substrate temperature (>400°C). The ITO films are policrystaline and crystallize in a cube bixbyite structure (In_2O_3). [7] The peaks observed for 2θ angle associated to the planes (222) and (400) may be observed in great evidence (Figure 3). The preferential growth of the ITO films according to the diffractogram is the (222) plane, and this orientation should be dependent on the deposition temperature. [8]

It was observed that the resisistivity of the prepared ITO film decreased significativilly (15 times) by annealing in nitrogen than in air atmosphere. The resistivity of $5.6.10^{-4}$ (Ω.m) has been obtained with thickness of 100 nm by van der Pauw method indicating that the ITO thin films obtained in this work are adequate for their use as biosensors electrodes.

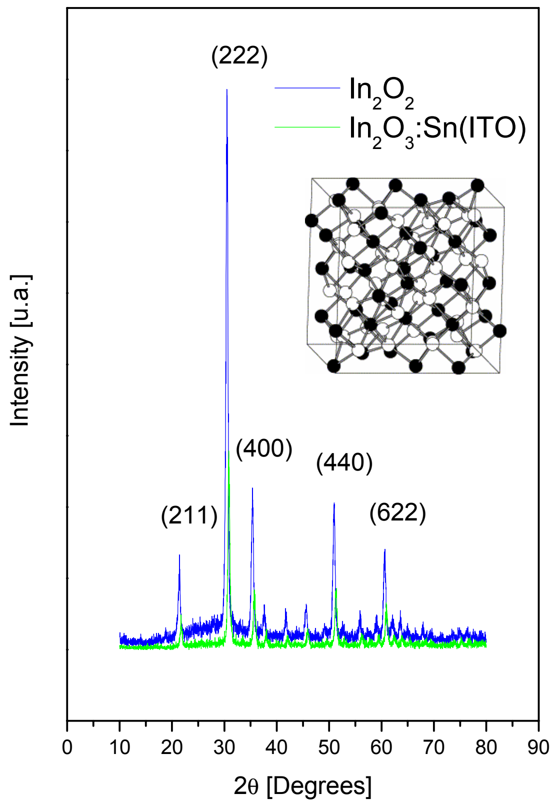
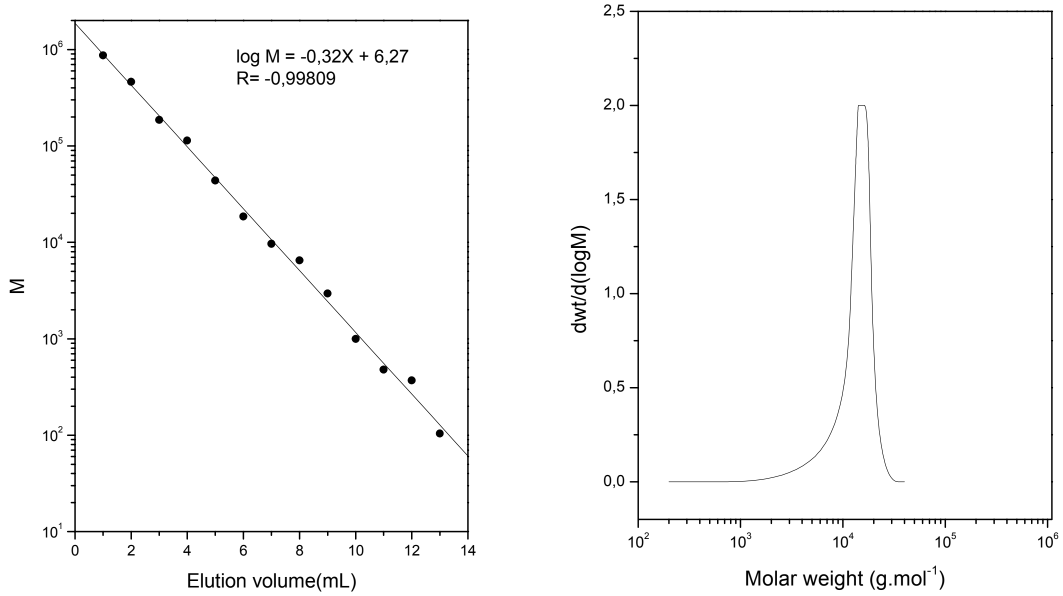


Figure 3: X-ray diffraction of ITO film deposited onto glass substrate. (blue: In_2O_3 and green: In_2O:Sn). In the figure, the bixbyite structure: black, indium atoms and white, oxygen atoms.

The Figure 4 shows the GPC analysis of the PAMAM (G4) obtained in this work. The peak observed in the figure confirms the dendrimer purity. A monomodal weight distribution and a low dispersion of the weight (M_n/M_w=1.15) is characteristic of the dendritic PAMAM structure.

The Figure 5 shows FTIR spectra between 4000 to 500 cm^{-1} for PAMAM generation 4. The peaks in 1645 cm^{-1} and 1557 cm^{-1} evidencing the presence of primary and secundary amines respectively, indicating there are more free NH_2 groups present on the periphery of the hight gereration PAMAM dendrimer (G4). This groups is important for enzyme immobilizations through the covalent bond between the NH_2 present in PAMAM and NH_2 of the enzyme (GOx).



(A) (B)

Figure 4: Calibration curve (A) and the GPC analysis of the PAMAM G4 (B).

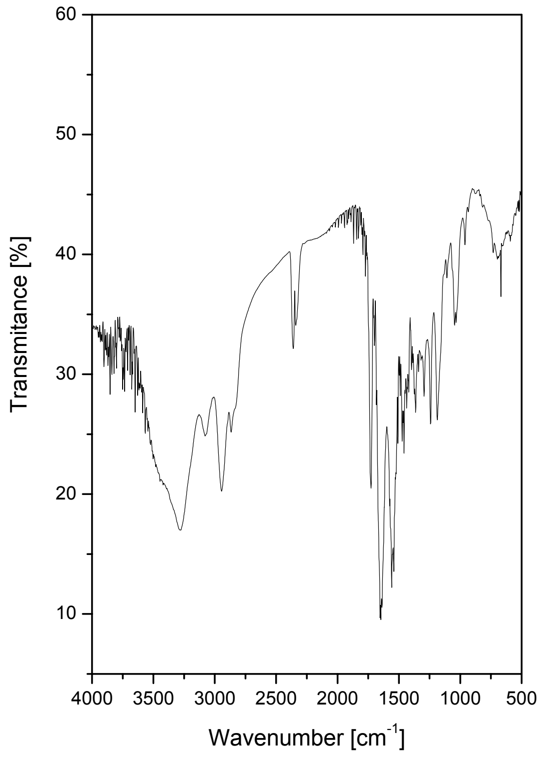


Figure 5: FTIR spectra of the PAMAM dendrimer (G4).

The IxV curve for PAMAM G4 is shows in Figure 6. The lower conductivity observed could be associated to the high dendrimers generation (G4).

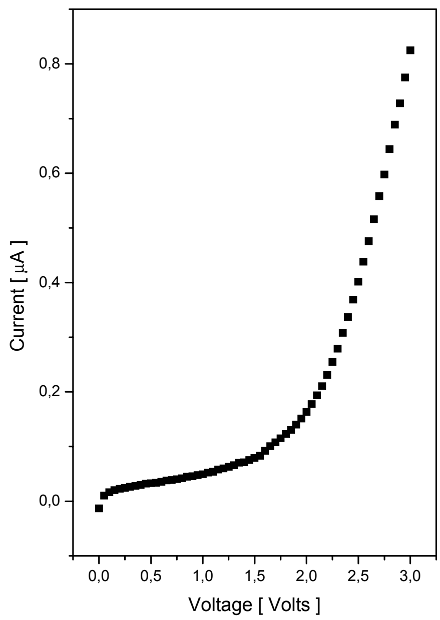


Figure 6: IxV curve for PAMAM dendrimer (G=4) at 25 ^{o}C.

To better understand the conductivity mechanism in the PAMAM/ITO films, we have made a molecular simulation using PM3 semi-empirical calculations (Hyperchem 7.0) and the energy of the dendrimer was minimized with molecular mechanics.

The electrostatic potentials for PAMAM (G0 and G1) are shown in Figure 7. There is more charge concentration in the dendrimer core region. The charge concentration on the core dendrimer increases with the increasing dendrimer generation evidencing a high potential barrier for the electrons-transfer from the core to the PAMAM dendrimer surface. [9-10]

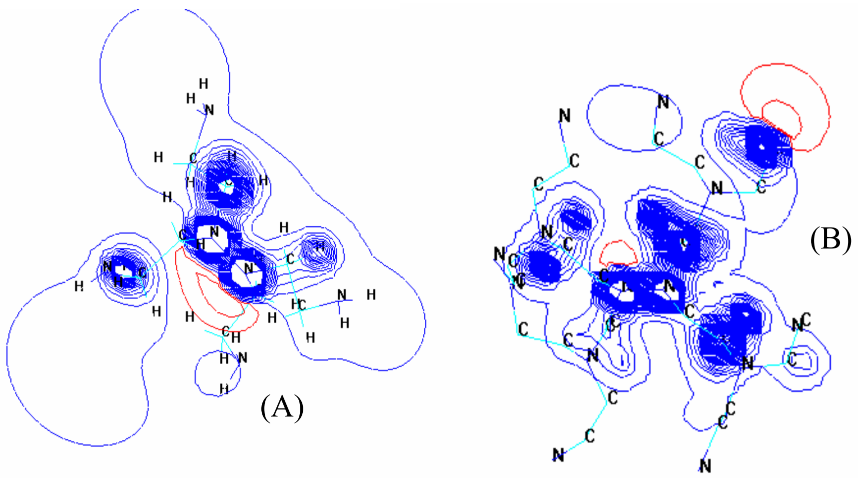


Figure 7: Electrostatic potentials for PAMAM G0 (A) and G1 (B).

The glucose sensing properties of the biosensor was analyzed by *in vitro* conditions tests. Figure 8a shows the biosensor signal variation with respected to the time at an anodic potential at 100 mV. The relationship between glucose concentration and response current at 100 mV potential is shown in Figure 8b. The relationship between glucose concentration and the electric current in the ITO/PAMAM-GOx-HRP biosensor may be expressed by the Equation (A):

$$\log(I) = 7.87.10^{-3}C - 7.04 \qquad \text{(A)}$$

where I is the measured current and C is the glucose concentration.

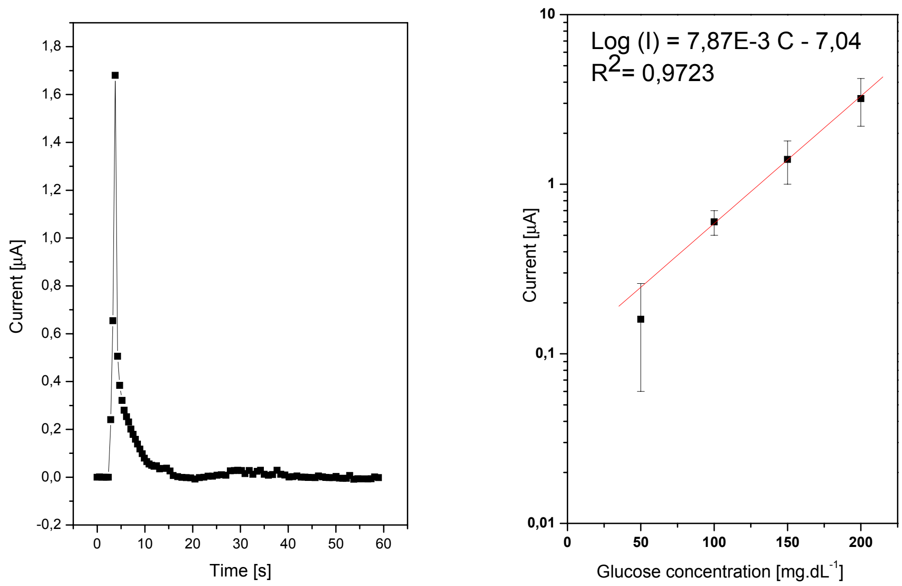


Figure 8: ITO/PAMAM-GOx-HRP amperometric biosensor response (100 mg.dL^{-1}) (a) and the relation between the maximum current and the glucose concentration. Applicated Anodic potential: 100 mV.

4. CONCLUSION

Smooth and transparent ITO thin films were obtained by the CVD technique. The results evidence that CVD technique is ideal for to synthesis of ITO thin films with good electro-optical properties. The optical properties of ITO and PAMAM dendrimers have been investigated and the results indicate the obtention of electrodes with lower band gap.

Poly(amido amine) dendrimer (PAMAM) of generation 4 was synthesized by divergent route and characterized by FTIR spectroscopy and GPC. Molecular orbital calculations suggested that the electronic transport through the PAMAM is dependent of the generation number of the dendrimer. The obtained ITO/PAMAM-GOx-HRP biosensor exhibited good stability, reproducibility and high sensitivity for the determination of glucose, which is important for clinical application.

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