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TITANIUM DIOXIDE NANOTUBES ON SILICON WAFER DESIGNATED FOR GOX ENZYMES IMMOBILIZATION

C. RAVARIU[a*], E. MANEA[b], C. PARVULESCU[b], F. BABARADA[a], A. POPESCU[b]
[a]*Faculty of Electronics, University Politehnica, Splaiul Independentei 313, Bucharest, Romania*
[b]*Institute of Microtechnology, Str. Erou Iancu Nicolae 32B, Bucharest, Romania*

The down-scaling of the integrated biosensors demands new methods for the enzyme immobilization, using nanostructured materials. This work presents the preparation method for the TiO2 nanotube by anodization and its applications to enzyme entrapping. Two kinds of electrolytes were investigated: oily and aqueous. When the electrolyte is glycerin the achieved TiO2 nanotubes are crown in some individual fasciculus, with weaker inter-links. For the glucose-oxidase enzyme immobilization, nafion is used as crosslink polymer. The surface characterization is operated by FEG SEM analysis.

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1. Introduction

The synthesis of the nanostructured materials is one of the challenges among nanotechnologies, [1]. The applications of these nanomaterials cover a large spectrum, especially in bioscience: drug delivery [2, 3, 4], nanomedicine [5], cell growth [6], or optical properties [7]. A dedicated section concerns nanostructured materials compatible with the Si-technology, in order to make possible the integrated biosensors manufacturing, [8]. One of the immobilization methods for the biodetecting layers uses nanoporous materials as adherent substrates. The enzymatic membranes are entrapped in organic compounds, [9]. On the other hand, titanium is a prevalent material used in prosthesis due to its bio-compatibility with the living matter, [10]. This paper presents the electro-chemical method for the TiO_2 nanotube grown on a Si-wafer and its applications in biosensors technology. The influence of the electrolyte composition on the pores nanostructure and morphology of the titanium dioxide layer, were investigated by SEM.

2. Experimental set-up

Among the TiO_2 layers growth methods are the sol-gel deposition [11] or oxidation by anodization of a thin Ti film, [12]. Although the oxidation of thin folium of titanium offers in literature many processing solutions, these methods are difficult to be applied to silicon integrated micro and nano-electronic devices.

In this section are described the preparation condition for a TiO_2 layer manufactured by anodization onto a silicon substrate. In order to ensure the compatibility with the Si-technology, the Titanium dioxide nanotubes growth, firstly imposes a thin film deposition.

Anodization stands for a simple and low cost method to synthesize TiO_2 by electrochemical oxidation of a metallic Ti film, deposited on a silicon wafer. In this way, a Ti-film,

* Corresponding author: cr682003@yahoo.com

over 90nm, was deposited by sputtering on a p-type Si-substrate with 1-2Ωcm resistivity. During this process, the argon gas flow was 2.5 sccm, while the base chamber pressure was 1.82mPa.

Then, the Ti metal, was converted into TiO_2 nanostructured material, by anodization. The electrochemical cell consists of three electrodes: a platinum sheet 7x10 cm^2 with a Pt wire used as a counter electrode, a standard calomel ($Hg/HgCl_2$) as a reference electrode and a work electrode composed by a Teflon sealing holder with a direct voltage contact applied on the titanium surface film. The work electrode (anode) and the counter electrode (cathode) were placed at 2÷4cm distance in an electrolyte solution. During the anodization, the potential drop was varied from 5V to 30V from an open circuit source, OCS, with a variable rate 0.1 ÷ 0.5 V/s. The optimal pH was kept between 6.4÷7.2.

Before anodization, the samples were degreased in acetone using an ultrasonic bath for four minutes and then rinsed in de-ionized water. After anodization, the samples were immediately washed with a large amount of de ionized water and subsequently dried in nitrogen atmosphere.

The electrolyte was sometimes oxalic acid and phosphoric acid as aqueous solutions (type-1), and other times salts in glycerin (type-2).

Immediately after the anodization, the TiO_2 layers are amorphous.

The morphology of the nanostructured TiO_2 was analyzed by a Field Emission Gun Scanning Electron Microscopy (FEG SEM), one of the latest version of SEM technology that combine the thermal emission technology using a newly developed Energy and angle selective Backscattered detector (EsB).

3. Results

3.1. The TiO_2 growth by anodization

Because the TiO_2 layers are amorphous after anodization, the samples were treated at different temperatures, in order to convert the oxide in a crystalline form. For instance, TiO_2 anatase phase results, after annealing at 773K. The fabricated structures present vertical nanotubes, with 100 - 400 nm width and a homogenous distribution.

Figure 1 presents the TiO_2 nanotubes developed on the Si-wafer, when the electrolyte was sodium sulfate, glycerin, water and sodium fluoride 0.4wt%, at 30V, so type-2. The morphology is similar with that achieved for completely aqueous electrolytes (type-1).

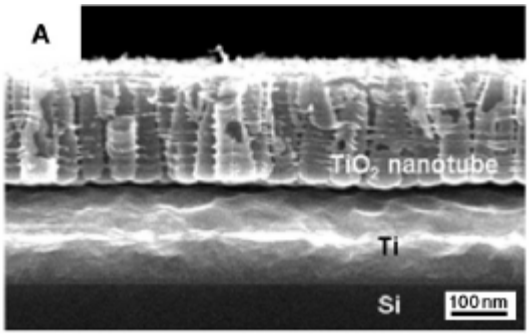

Fig. 1. FEG SEM images in a cross section.

The FEG SEM analysis revealed that the morphology of the TiO_2 nanotubes is depending on the electrolyte formula. Figure 2 presents the FEG SEM image of a TiO_2 nanotubes layer made in completely oily glycerin solution of electrolyte (ammonium fluoride in glycerin), at 10V anodization voltage. The nanotubes are crown in some individual fasciculus, with weaker inter-links.

The best morphology of the TiO_2 nanotubes is achieved when the anodization occurs in aqueous electrolytes, versus the case when the solvent is the glycerin. The most probable explanation is related by the ions mobility in aqueous and oily solutions, [13].

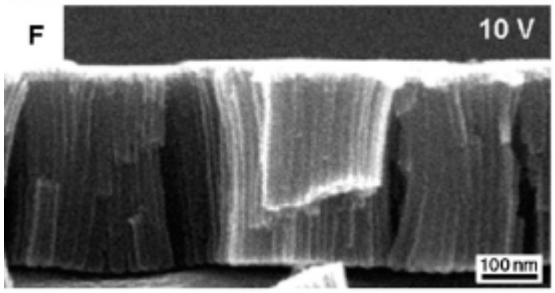

Fig. 2. Lateral view by FEG SEM of a TiO_2 nanotubes layer anodized at 10V.

Therefore, the diffusion rate of the ions in water is superior to that in glycerin, with an increased growth rate in this first environment.

3.2. Enzyme entrapping

The TiO_2 nanostructured material was grown on the Si-wafer, in order to offer a bio-compatible inorganic support for enzymes entrapping, like glucose-oxidase, GOX. The surface characterization is continuing to be ensured by SEM.

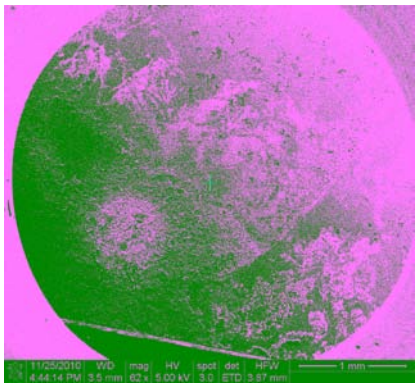

Fig. 3. SEM image of the probe with: glucozoxidase / nafion / TiO_2 / SiO_2 / Si.

Usually, the GOX entrapment on a solid surface, is possible via the cross-link method, using a glutaraldehyde solution (GA) - 2,5% concentration, as polymerization agent and serum albumin from bovine provenience (BSA), [14]. In these tests, another polymer was researched - nafion - in the same concentration as the glutaraldehyde. All the time, a buffer solution ensures a constant pH=7.0. We use nafion - that is a fluoropolymer-copolymer based on sulfonated tetrafluoroethylene because it is presenting an excellent mechanical and thermal stability, [15].

Fig. 3 presents the SEM image with the successive films from the Si-substrate up to GOX enzyme. The GOX membrane adheres to the substrate, but is still scanty to the homogeneity. A possible improvement could be the increasing of the nafion quantity.

4. Discussion

The first instance for the enzymatic membrane characterization placed on nanoporous TiO2 appeals to the probe spectrum.

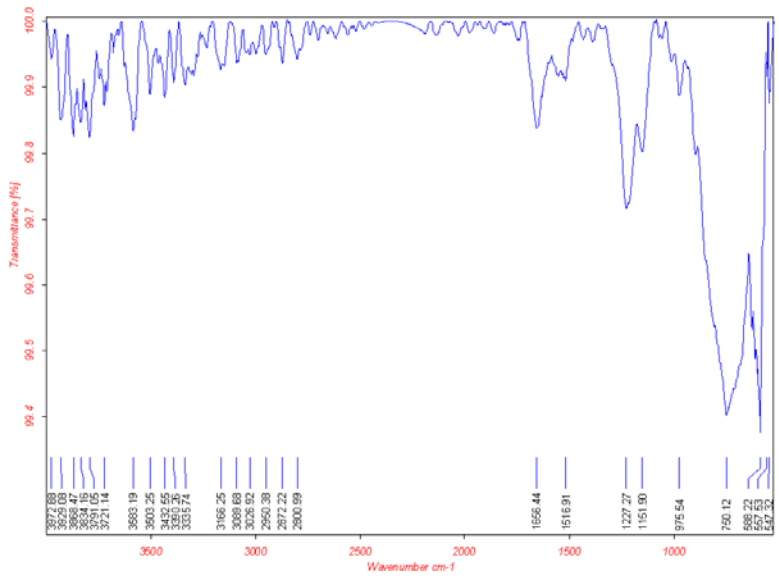

Fig. 4. The probe spectrum: glucose-oxidase + nafion +TiO_2/SiO_2/Si.

In figure 4 is presented a probe spectrum after GOX immobilization in nafion. The water spectral bands interfers with target bands and make difficult the interpretation. After, the water bands extraction, two bands can be observed, with the following explanation: I-st amide (C=O), 1656 cm^{-1} and respectively II-nd amide (N-H), 1517 cm^{-1} from glucose-oxidase. The bands from 1227 cm^{-1} and 1152 cm^{-1} can be atributed to the vibration mode for C-O or C-F, signifying the nafion bounds. In the 750÷550 cm^{-1} domain, can be observed characteristic bands associated to the Ti-O bound. They are deviated bands comparatively to the substrate spectrum that suggest a TiO2 anchoring to substrate, establishing Ti-O-C bounds in 976 cm^{-1} spectrum.

5. Conclusions

A nanostructured titanium dioxide on silicon was manufactured. The best TiO_2 nanotubes were manufactured by an anodization process at potentials of 5÷10V, maintaining a pH of 6.4÷7.4, preferably acid. The quantity of solvent must be enough in order to let free the anions and cations in solution, taken into account that the upper limit concentration of the (NH_4F) salt in water is 40%, for saturated solution. An experimental study proofed different TiO_2 morphologies for aqueous electrolyte or glycerin.

The TiO_2 nanotube layer has a double advantage for the glucose-oxidase immobilization: high enough adsorbent properties for the GOD enzyme and good electro-oxidation catalytic properties for some organic substances, due to its biocompatibility with the living matter.

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