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Spectroscopy studies of functionalized oxidized porous silicon surface for biosensing applications

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ABSTRACT

In this paper we report detailed Fourier transform infra-red (FTIR) and Raman spectroscopic results obtained after the modification of a porous silica surface using different steps of functionalization and protein grafting. After the elaboration of porous silicon (PS) layers, with sufficient adjusted pore size and well defined porosity, we carried out complete thermal oxidation and we optimized the silanization step by varying the concentration of 3-aminopropyltriethoxysilane (APTES) molecules using different immersion and rinsing durations. Then we introduced a coupling agent, glutaraldehyde (GL) molecules, which has a great affinity for bovine serum albumin (BSA) molecule grafting. FTIR and Raman spectroscopic analyses present complementary spectra that allow us to get an idea about the chemical links and vibrational modes that appear during the functionalization process and after protein attachment. This proves that the biological molecules are covalently attached on the modified porous silica surface.

Moreover, a modelling study of the reflectance spectra allows values of the volume fraction and refractive index variations to be estimated by assigning them with the number of APTES, glutaraldehyde and protein layers after each step. These results are well correlated to those obtained by the FTIR and Raman analyses.

This work on porous silica single-layers is carried out in order to exploit it for the elaboration of functionalized optical waveguides to obtain a sensitive label-free optical biosensor.

1. Introduction

Recent optical biosensors are used for bio-molecular analyses in new integrated bio-analytical systems in medical diagnostics and health care, monitoring of the environment, control of food products and their use in the pharmaceutics industry. Research into biosensors has experienced a noticeable development over the last few years especially in label free optical biosensors [1–3]. This kind of biosensors does not need radioactive or fluorescent labels to be used. The biosensor is primarily constituted by the biological recognition system and the transducer element. The biological recognition system allows the identification of the analyte through its site by providing selective molecular recognition. The transducer element is a device which converts the molecular event into a physical measurable signal. Nowadays, there are continuously increasing research studies to find new methods and devices that would provide easy, reproducible and sensitive sensing assays for bio-molecular reactions. Many label free optical biosensors based on porous silicon (PS) have been developed such as Bragg mirrors [4,5], waveguides [6–8] and microcavities [9–11]. PS is an attractive promising material for biosensing because it is cheap, easy to obtain and it has a porous structure with a large specific sensing area (up to 800 $m^2 g^{-1}$) [12]. Furthermore, this last property makes PS very sensitive to the presence of biochemical elements which penetrate inside the pores by immobilizing bio-molecules. These characteristics make it an ideal optical biosensor material. However, the surface of PS is strongly hydrophobic so that the aqueous solution cannot infiltrate into the pores. Thermal oxidation and chemical adaptation must be applied respectively to stabilize and functionalize the internal surface of the porous silicon layer. This step is essential to obtain subsequent efficient molecules attachment. Moreover, total oxidation is necessary to obtain transparent porous silica for waveguide biosensor applications.

This work is divided into two complementary parts. Firstly, we present a detailed study of the silanization step. The modification of the oxidized porous silicon surface was investigated by Fourier-transform infrared (FTIR) and Raman spectroscopy techniques after each step of chemical functionalization and protein grafting with

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molecules like bovine serum albumin as a test molecule. Secondly, we show the evolution of the volume fraction and refractive index after each step using an effective medium approximation model.

2. Experimental

2.1. Preparation of PS samples

Porous silicon (PS) samples were prepared by electrochemical anodization of (100)-oriented highly doped p-type silicon substrate (7 mΩ cm). The electrolyte used was composed of HF (50%)–ethanol–H_2O (deionised water at 18 MΩ, DIW) solution with the ratios of 2–2–1 respectively. The anodic current density of 50 mA cm^{-2} was applied during 120 s. The anodization parameters have been chosen in order to obtain PS layers with a porosity of about 70% (value determined by fitting the optical reflectivity data) and a thickness of 4 μm (measured by SEM). The anodization process was carried out in a Teflon electrochemical cell at room temperature and in darkness.

2.2. Functionalization of PS samples

Freshly porous silicon layers are unstable in relation to the attachment of bio-molecules purposes and their contact with air generates a change in the PS properties over time [13]. In order to stabilize the PS surface and also as a prerequisite step for the chemical functionalization, the PS layers are oxidized by a heating treatment for 1 h at 950 °C in wet O_2, resulting in completely oxidized porous silicon (OPS) layers. After oxidation, the mean pore diameter is around 35 nm. In this case the transparent OPS in the visible range is suitable for low loss optical waveguides.

After this thermal treatment, the surface must be activated with silanol groups [14]. Procedures for the immobilization of bio-molecules are usually based on a chemical reaction which involves the silanization of the OPS surface [15]. First, OPS samples were immersed in various concentrations of 3-aminopropyltriethoxysilane (APTES, from Sigma–Aldrich) and a hydro-alcoholic mixture of DIW and methanol (1–1) for 20 min at room temperature. Then, the samples were thoroughly rinsed several times with DIW, dried with N_2 and heated at 100 °C over 10 min to evacuate the solvent vapour.

In the second step of the chemical functionalization, the samples were immersed in 2.5% solution of glutaraldehyde (GL, from Sigma–Aldrich) adjusted to pH 7 using sodium hydroxide solution for 1 h at room temperature. Then the samples were cleaned with a solution of Tween 20 in phosphate buffered saline (PBS, from Fluka) and with DIW in order to remove any excess GL molecules. APTES and GL are efficient coupling agents that promote adhesion between the OPS surface and bio-molecules. Furthermore, APTES and GL molecules have small size with a diameter of 0.8 nm and 0.7 nm respectively that can infiltrate easily into the pores and form uniform thin layers on the internal surface of OPS.

Finally, functionalized OPS samples were immersed in a solution of bovine serum albumin (BSA, from Sigma–Aldrich) for 2 days at room temperature. The dimensions of BSA are 3 nm × 8 nm × 8 nm [16]. The BSA solution was prepared by dissolving 30 mg of BSA powder in 10 ml of DIW. After this impregnation, the OPS samples were taken out of the BSA solution, rinsed with DIW and dried under a steady nitrogen gas flow. The schematic representation of the OPS surface modification after each step is shown in Fig. 1.

2.3. Material characterization

2.3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopic analysis was used in order to characterize the presence of specific chemical groups bonded to the chemically modified surfaces after each step. FTIR spectra were obtained in the wavenumber range from 650 to 4000 cm^{-1}. All spectra were collected by an FTIR spectrometer (Perkin Elmer Spectrum 100) with 4 scans and 4 cm^{-1} resolution. Almost all vibration bands were identified.

2.3.2. Raman spectroscopy

Raman spectra were recorded with a HORIBA Jobin Yvon Micro-Raman spectrometer (LabRAM HR) using an Argon laser excitation wavelength of 488 nm with a power of 47 mW or an He–Ne laser excitation wavelength of 633 nm with a power of 7 mW at the sample. The room temperature Raman spectra were collected through a confocal optical microscope, coupled to a holographic grating (600 grooves mm^{-1}) with a resolution less than 1 cm^{-1} by a CCD camera detector.

2.3.3. UV–Vis–NIR spectroscopy

The instrumentation used for the measurements of all optical reflectance spectra was based on the spectroscopy system (Ocean Optics HR 4000). The optical system was equipped with a Tungsten Halogen white source (HL2000, wavelength range 360–2000 nm), a monochromator (wavelength range 200–1100 nm) and a bifurcated optical fiber. All reflectance spectra were obtained and investigated in the 600–1050 nm wavelength range at ambient temperature.

2.4. Refractive index extraction by modelling the reflectance of a porous silica layer

The method of determining the refractive index of functionalized and protein attachment OPS is based on the adjustment of the experimental reflectance spectra with the theoretical ones. Functionalized OPS is an effective medium constituted by air, silica and any other material which can be introduced into pores. In order to determine the refractive index of such a medium, we used the model of Bruggemann [17] which links the refractive index constituents to their volume fraction C_i with the following relationship:

$$\sum_i C_i \frac{n_i^2 - n^2}{n_i^2 + 2n^2} = 0 \quad \text{with} \quad \sum_i C_i = 1$$

where n_i and n are the refractive index of the constituent i and the effective medium respectively.

The model of Bruggemann was applied after each step, taking into account the refractive index and the volume fraction for each constituent (air, silica, APTES, GL and BSA) (Fig. 2).

3. Results and discussion

The silanization of OPS structures in the liquid phase is crucial with regard to the efficiency and reproducibility of the chemical functionalization. So, preliminary experiments were carried out. The purpose of this study is to find the optimum silanization conditions to obtain single layer of APTES on silica. Therefore, aggregation

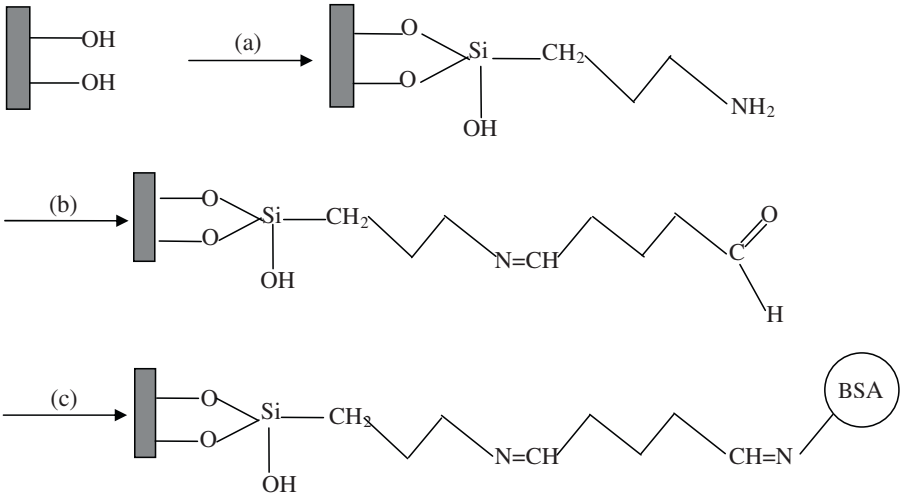

Fig. 1. Schematic representation of the OPS surface modification: (a) silanization with 3-aminopropyltriethoxysilane (APTES), (b) introduction of glutaraldehyde (GL) as a coupling agent, (c) grafting of BSA.