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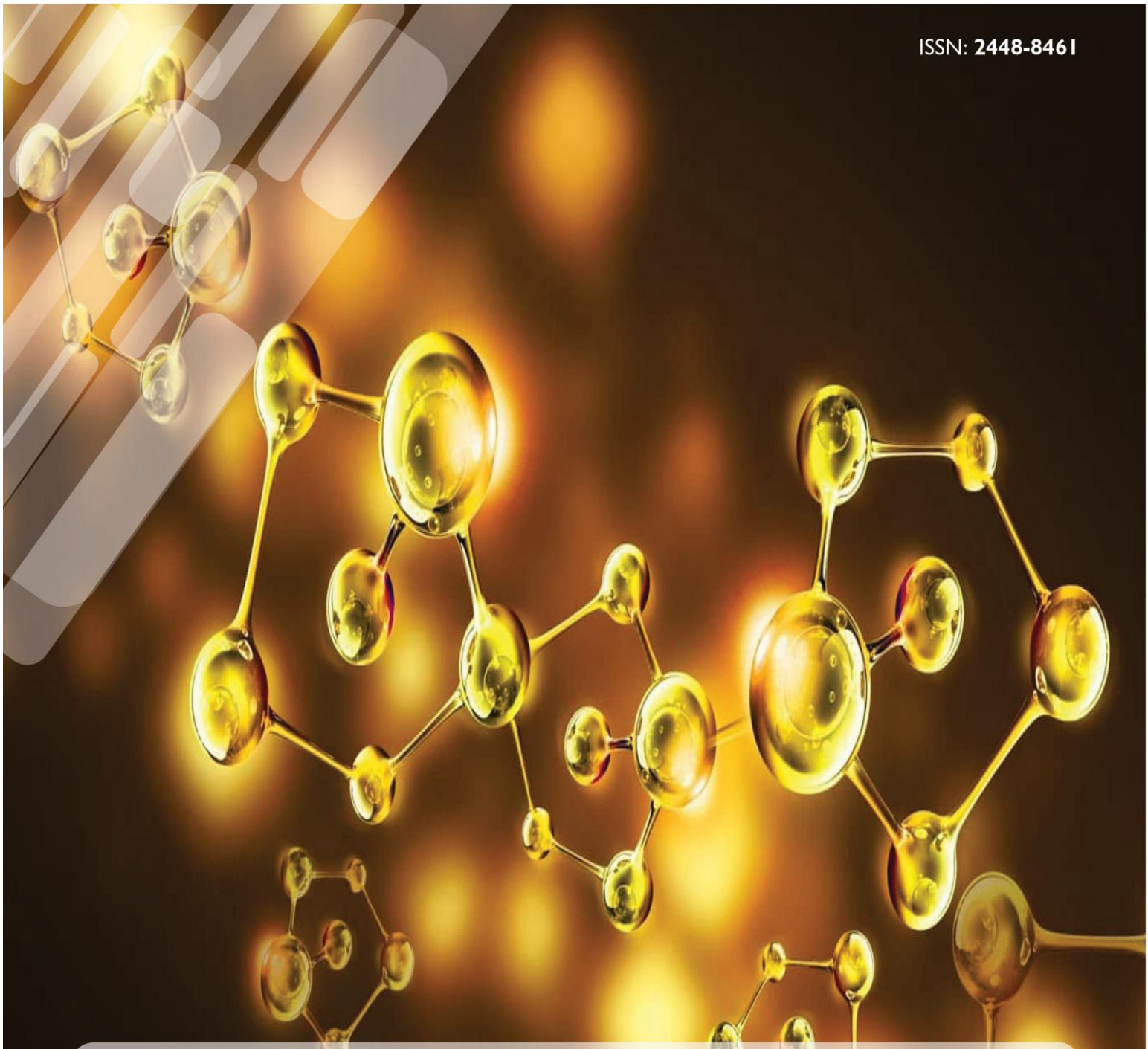

Porous Silicon Based Biosensor for Detection of Biogenic Amine (Spermidine)

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Resumen

Estructuras de silicio poroso (SP) con nanopartículas de oro (AuNPs) se obtuvieron mediante ataque electroquímico asistido con sal metálica (ácido HAuCl4). La alta superficie de la matriz de SP y las AuNPs permitieron incrementar la sensibilidad del biosensor. Esta estructura se usó como plataforma para obtener un biosensor de aminas biogénicas (AB). La detección óptica de AB se basó en los cambios de las señales relacionadas con las reacciones entre la AB y el elemento reconocido utilizado (diamina oxidasa). En este trabajo, el SP con AuNPs se bio-funcionalizó mediante la introducción de grupos funcionales en su superficie mediante el método de adsorción. La Microscopía Electrónica de Barrido (SEM) y Espectroscopia de Dispersión de Energía de Rayos X (EDS) mostraron la introducción de AuNPs en la estructura del SP. Los estudios de espectrometría infrarroja con Transformada de Fourier (FTIR) demostraron la correcta introducción de los elementos del biosensor. El potencial de las superficies de SP bio-funcionalizadas se estableció para ser utilizado como biosensor en la detección de AB.

Abstract

Porous silicon (PS) structures with gold nanoparticles (AuNPs) were obtained by electrochemical etching assisted with metallic salt. High surface matrix of the PS and the AuNPs would allowed the increment in the sensitivity of the biosensor. This structure was used as platform to get a biogenic amine (BA) biosensor. The optical detection of BA was based on the changes of the signals related to the reactions between the BA and the recognized element used (diamine oxidase). In this work, PS with AuNPs was bio-funcionalized through the introduction of functional groups on its surface by adsorption method. Scanning Electron Microscopy (SEM) and X Ray Energy Dispersive Spectroscopy (EDS) showed the introduction of AuNPs in the PS structure. Infrared spectrometry with Fourier Transform (FTIR) studies demonstrated the correct introduction of biosensor elements. The potential of the bio-functionalized PS surfaces was established to be used as a biosensor in the detection of BA.

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1 Introduction

Porous silicon (PS) is a very promising material due to its chemical (Coffer, et al, 2005) and optical properties (F. Severiano, et al, 2014). These porous structures are excellent candidates for devices in the fields of electronics (Lehmann, et al, 1991), optoelectronics (F. Severiano, et al, 2014), biochemical (Coffer, et al, 2005), as well as biosensors (Chattopadhyay, et al, 2002). PS can be obtained by electrochemical etching in a solution of hydrofluoric acid (HF) and ethanol. The characteristics of the porous layer (pore diameter and thickness of the porous layer), can be easily controlled by the etching parameters (DeStefano, et al, 2004). The internal surface of PS matrix is hydrogen-terminated after the etching, which allows immobilize biomolecules on the whole porous surface (Mathew and Alocilja, 2005). Due to its characteristics, PS can be applied as a platform in the obtaining of optical or electronic biosensors. On the other hand, Au nanoparticles (AuNPs) have been used as catalyst (Lopez-Sanchez, et al, 2011), image contrast agents (Alkilany, et al, 2013), biosensor and bioanalytical element (Sassolas, et al, 2008) and meaningfuly as part in electrochemical detection platform. This is due to its properties: large surface area, scattering and absorption of visible light, high density of electrons and catalytic properties (Willner, et al, 2010). With the incorporation of AuNPs in the PS structure it sought to increase the sensibility of biosensors (Lars, et al 1992). One of the areas where the application of biosensors is essential, is in the food industry, especially in the chain of quality control, since in recent years biogenic amines (BA) have been related with food intoxications, (Leuschner, et al, 1998), because of their psychoactive or vasoactive effect as well as their use as quality standard in food. Among the symptoms that the BA produce are: skin rash, epigastric pain, disturbances of the gastrointestinal tract; this kind of health problems are treated with antihistamines. For these reasons BA are used as food quality indicators. BA are produced by microbial decomposition in food with high content of proteins. Also the processing, maturation and storage of foodstuff (fish, meat, cheese, beer, wine, etc.) are important factors in the BA generation (Leuschner, et al, 1998). Diamines such as putrescine, cadaverine and histamine are decomposition products of lysine, ornithine and histidine respectively. The intake of large quantities of histamine could lead to scombrotoxicosis, while other BA are related to effects in respiratory distress, nausea, hyper or hypotension, as it was mentioned before. For these reason is important to develop BA biosensors with high sensitivity. Actually there are different methods to measure the presence of BA, for example, histamine has been sensed by derivatisation with fluorescent reagents followed by chromatographic separation (Lopez-Sabater, et al, 1993). This method might be tedious and required trained personal, besides of expensive equipment. Other methods to measure the presence of BA are immunochemical, capillary electrophoresis and gas chromatographic methods, all these methods required long time of analysis and expensive equipment. To reduce the time of BA sensing, some enzymatic methods and enzyme biosensors have been applied (Lopez-Sabater, et al, 1993; Chemnitius, et al, 1992). This kind of biosensor show advantages such as rapid analysis and few sample preparation. Consequently the main objective of this work was the attainment of a BA biosensor based on enzymes. The objective of the enzymes was the immobilization of the BA, and the introduction of AuNPs in the PS structure was to improve the sensibility of the biosensor. For this work it was used diamine oxidase (DAO) from pig kidney to the enzymatic immobilization of BA. Finally, this biosensor based on DAO, allowed the measure of spermidine.

2. Materials and methods

2.1 PS with AuNPs

P-type silicon wafer with a resistivity of 5-10 Ω-cm were used to obtain PS with AuNPs. The method used was metal salt-assisted chemical etching (F. Severiano, et al, 2017). This process was carried out in a Teflon cell designed with the habitual setup to obtain PS. The electrolyte was composed of a mixture of hydrofluoric acid (HF, Merck 40% by volume), ethanol (Alfa-Aesar 99.98% by volume) and cloroauric acid ($HAuCl_4$ 99.99% trace metals basis, diluted at 30 wt. % in HCl (Aldrich)). The metal salt was prepared at ratio 1mM and then it was incorporated to the electrolyte. The volume ratios used in the electrolyte were 1/2/1, HF/ethanol/HAuCl4 respectively, and the etching time was about 2 hours.

2.2 PS/Au nanoparticles functionalization (silanization)

The silanization of PS/AuNPs was carry out using (3-aminopropyl) trimethoxysilane (APTMS). APTMS was diluted in toluene pre-heated between 100-200 °C. The solution was prepared at 2% of APTMS in volume. The PS/AuNPs samples were then immersed in the solution for 3 hours at room temperature. The substrates were then removed from the solution, rinsed with toluene and dried in an oven at 100°C for 1 hour.

2.3 Preparation of a bio receptive surface

A covalent coupling of DAO (from pig kidney) on functionalized PS/AuNPs was performed via standard amine coupling chemistry with the first step being an activation of the surface by a mixture of 0.043g of N-hydroxysuccinimide (NHS) and 0.035g of N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydro-chloride (EDC) dissolved in 20 ml of phosphate buffered saline (PBS). This solution was agitated for 15 minutes and filtered (filter paper of 0.20 μm).

Functionalized PS/AuNPs samples substrates were immersed for 20 minutes at room temperature. The substrates were removed and rinsed with PBS.

2.4 Incorporation of DAO (Recognition Element)

Diamine oxidase (DAO) from pig kidney was used to the enzymatic immobilization of BA which was introduced by adsorption. 20 mg of DAO were dissolved in 500 μL of PBS. Then 300 μL were put on the surface of the bio receptive surface (PS/AuNPs/silanization/EDC and NHS) and they were left for 2 hours. After that, the substrate was rinsed with PBS.

2.5 Sensing of BA

The sensing process of the biosensor was carried out through FTIR spectroscopy: attenuated total reflectance (ATR) mode. The BA to sense were dissolved in PBS and put in contact with the biosensor. The interaction kinetics was analyzed for 30 minutes, each spectrum was taken every 30 seconds.

2.6 Scanning electron microscopy (SEM)

Superficial and cross section images of PS/AuNPs platform were obtained using a high-resolution field emission SEM Jeol, model JSM 7800F. X ray Energy Dispersive Spectrometer (EDS, model Apollo XL) was used to chemical composition analysis.

2.7 Fourier transform infrared spectroscopy (FTIR)

The biosensor was characterized by FTIR in ATR mode. FTIR spectra were recorded with a Vertex 70 (Bruker) spectrometer, and each spectrum was the average of 30 repetitions.

3. Results and discussion

3.1 Array of the biosensor

Figure 1 shows the graphical representation of all steps taken during the biosensor construction. From left to right: a) Formation of PS with AuNPs platform, b) silanization, c) preparation of a bio receptive surface (EDC and NHS), d) introduction of recognition element (DAO), e) process of sensing of BA.

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3.2 Microstructure of PS/AuNPs from SEM and EDS analysis

The platform of the biosensor (PS/AuNPs) was characterized by electronic microscopy. Figure 2 shows SEM images of the surface of the sample obtained, 5000X and 20000X are shown in the above and below in the left side respectively. For a better analysis of the distribution and quantity of the AuNPs in the porous silicon layer (PSL), SEM images composed with secondary and backscattering electrons were obtained, right side images. This analysis demonstrated the incorporation of AuNPs over and inside the porous structure. PS/AuNPs sample presented pores and cavities, the pore diameter for this samples was around 1.5 μm and the cavities 1.8 μm. AuNPs were on all the PS surface. The pore size and the presence of cavities can favor the introduction of the elements of the biosensor in the PSL.

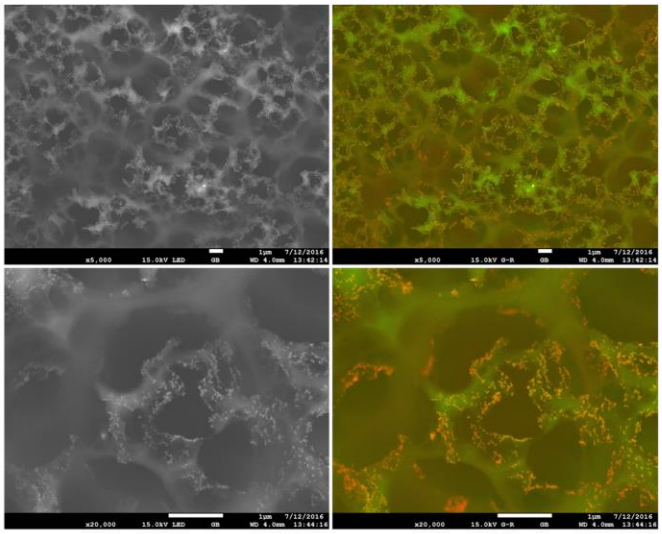

Figure 2. SEM images of PSL used as platform in biosensors. SEM images of 5000X and 20000X are shown in the left side. SEM images composed with secondary and backscattering electrons are shown in the right side.

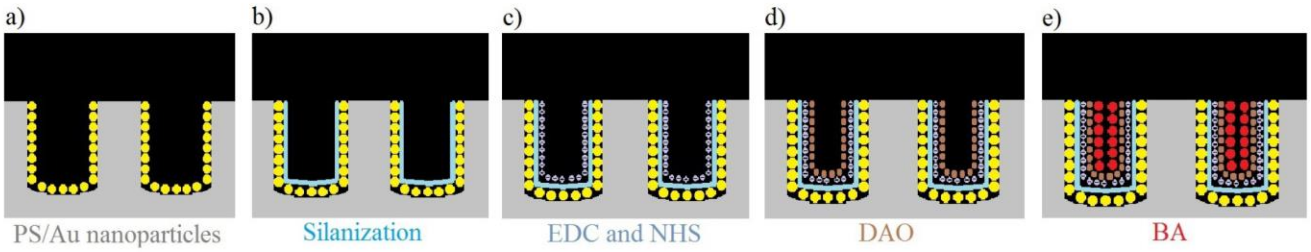

Figure 1. Array of the elements of the biosensor. a) PS with AuNPs platform, b) silanization, c) introduction of EDC and NHS, d) introduction of DAO, e) sensing of BA.

EDS analysis was done to know the chemical composition of the samples obtained with $HAuCl_4$, figure 3. The EDS results showed the incorporation of Au (peak at 2.12 keV) in the PS structure. Particularly, the X ray emission lines corresponding at Au must appear between 2.16 - 3.0 keV. The more intense peaks correspond to $M_{\alpha 1}$, $M_{\alpha 2}$ $M_{\alpha 1}$ lines (between 2.12 and 2.25 keV), however it is known that there are important contributions of the M_y family and the X-ray absorption edges that causes the typical broadening of the M peak for heavy metals.

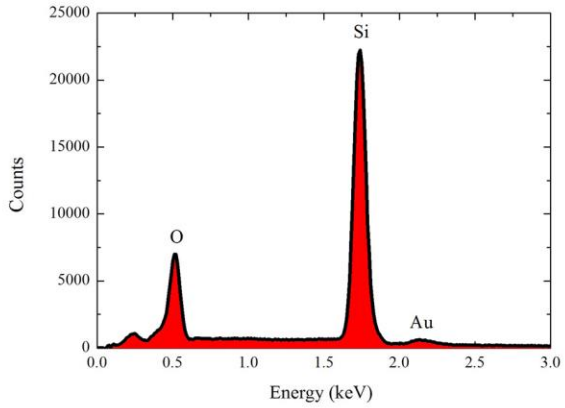

Figure 3. EDS spectrum that confirm the introduction of AuNPs in the PS.

3.3 FTIR characterization

All steps in the process of assembly of the biosensor was characterized through FTIR. Figure 4 shows the absorbance spectra of all steps conducted to obtain the platform of the biosensor. Step 1: Black line in figure 4 (black line), shows the spectra of absorbance for the platform of PS/AuNPs. The assignment of peaks was: 610 cm^{-1} was related to the bending of hydrides (SiH_2) and the vibrational modes at 1075 and 1200 cm^{-1} were due to the oxidation (Si-O-Si) in the PSL (Iler, 1979). This vibrational properties of silicon are well known. This characterization did not show peaks related to the AuNPs, but the images of SEM show the incorporation of Au in the surface of PS. Step 2: The process of silanization was realized after the obtaining of PS/AuNPs. The substrates (PS/AuNPs) was immersed in a solution at 2% of APTES in toluene. Absorbance spectra, figure 4 (red line), shown the PS/AuNPs after the process of silanization. The assignment of peaks and bands was: the band at 470 cm^{-1} was associated to vibrations of hydroxyl groups (Si-OH), the band that include the signals 1035 and 1127 cm^{-1} was due to the oxide as it was said before, since the vibrational modes of silicon oxide are included in the region from 950 to 1250 cm^{-1} (Queeney, et al, 2000; Queeney, et al, 2004). Vibrations at 1320, 1490 and 1636 cm^{-1} correspond to the symmetric and asymmetric -NH_{3+} deformation modes, this is an indicative of amine group protonation when the samples are exposed to air (Pasternack, et al, 2008). The NH_2 scissor vibration found at 1565 cm^{-1} confirms the presence of the terminal groups of the APTMS molecules. The results showed that the process of functionalization was successful. Step 3: The introduction of the linkers (EDC and NHS) was realized by adsorption, as was mentioned above. Figure 4 (blue line), shows the absorbance spectrum obtained after the EDC and NHS introduction. The assignment of peaks and bands was: the band at 550 cm^{-1} was associated to vibrations of hydroxyl groups (Si-OH). The band that include the signals 1066, 11500 and 1228 cm^{-1} was due to the oxide as it was said before. The peak at 1548 cm^{-1} was related to the amide II and is due to the bending of N-H and stretching of C-N in the plane. The peak at 1650 cm^{-1} (amide I) was due to the stretching of C=O and bending out the plane of C-N. The peaks at 1548 and 1650 cm^{-1} are related to the primary amines and secondary amines, respectively (Barth, et al, 2007). The peaks associated to the presence of amides prove the incorporation of the linkers in the surface of the silanized surface. Step 4: The introduction of the element of recognition was realized by adsorption from a drop of DAO diluted in PBS. Figure 4 (cyan line), shows the spectra of absorbance for this step of the process, it did not show changes in comparison with the previous step. This was due to the signal of the linkers (EDS and NHS), since the response of the DAO was expected around the 1630 and 1525 cm^{-1} (signals related to the amides). Due to this it was not seen the signal attributed to the presence of DAO. But with this structure the process of sensing was realized.

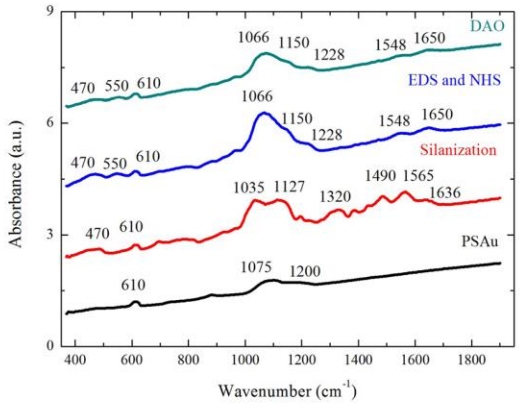

Figure 4. Spectra of absorbance of all steps applied during the construction of the biosensor.

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3.4 Sensing of biogenic amines

The characterization of the biosensors was carried out using ATR. The biosensors were made on PS/AuNPs platforms, which were silanized and later they were added linkers (EDS and HNS) and recognition (DAO) elements. The spermidine used in the sensing process were dissolved in buffer solution (TRIS). This solution was put in contact with the crystal used in the technique of ATR. The spectra were taken in a sequence of 60 spectra and each spectrum was composed of 30 iterations. The aim is observe the band associated to ammonia at 1132 cm^{-1}, this is due to the decomposition of the BA when enter in contact with the DAO. The spectra showed in the next sections are composed by a small number of measurements, in order that the increase of the signal of interest was clearly appreciated. Figure 5 shows the spectra obtained from the biosensor when enter in contact with spermidine. 20000 ppm were prepared in 1 milliliter and 20 microliters were taken for measurement. The biosensor showed response around 1132 cm-1, this response is associated to the ammonia,

a reaction product that took place when spermidine and DAO enter in contact, which confirm the BA presence.

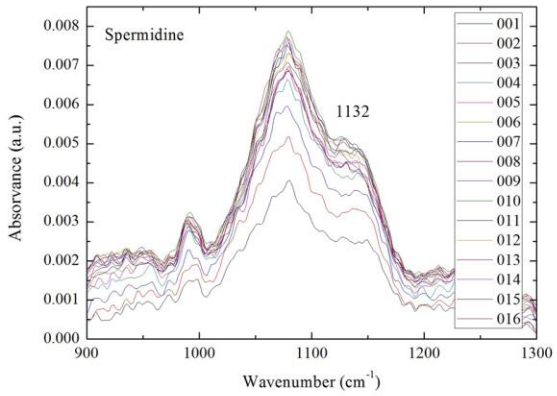

Figure 5. Infrared spectra obtained from biosensor when was put in contact with spermidine. The biosensor showed response around the region of interest (1132 cm^{-1}).

Conclusions

This study was conducted with the aimed to fabricate and effectively characterize a biosensor of BA. PS with AuNPs were obtained with electrochemical etching method using a metal salt in the electrolyte. The introduction of silanes, linkers and elements of recognition was proved and characterized with Fourier transform infrared spectroscopy. The biosensor was able of sensing Spermidine.

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