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Theoretical evaluation of a fiber-optic SPR biosensor based on a gold layer treated with thiol acid

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Abstract. In the present work, we have investigated the surface plasmons resonance (SPR) biosensor based on optical fiber applied for the detection of pathogenic bacteria (*Escherichia coli*), in order to overcome the stresses caused by the massive prism and to obtain a reliable and efficient miniature device. The modeling procedure is based on a matrix formalism developed for the optical studies of multilayer media. We have tested the response of the fiber optic biosensor based on a golden substrate functionalized with thiol acid. The results show that the SPR biosensor sensitivity is improved by treating the gold electrode with thiol. An improved SPR biosensor with a high resolution is obtained.

1 Introduction

Due to their high sensitivity, real-time response, and high accuracy, surface plasmon resonance (SPR) sensors are extensively used for the study of antigen/antibody interactions [1,2]. This type of biosensors continues to attract research interest and is increasingly used in a wide range of fields such as: biology, biochemistry, medicine, food industry and the environment [3–5]. These devices are based on the variation of the plasmonic response which is related to the evolution of the refractive index of the studied dielectric. SPR is an optical phenomenon in which light interacts with the electrons at the interface between a metal and a dielectric.

The SPR biosensors have been extraordinary developed in the field of detection, analysis of chemical or biological compounds and their interactions. Dudak and Boyaci [6] have studied the SPR biosensor technology used for the detection of bacteria and they have focused on the development of SPR immunoassays, in order to improve their instrumental sensitivity. They have predicted that in the near future, SPR biosensors will undoubtedly take their place on the commercial market. Jang et al. [5] have developed a fiber optic SPR biosensor to detect prostate specific antigen as a prostate cancer biosensor. SPR was studied by Green et al. [7] by showing the diversity of the SPR analysis to study the biological interaction and to use it as a tool for characterization of the biomaterials which allows to determine the dielectric properties.

Currently, various optical devices have been designed to create SPR. The most widespread configuration is that proposed by Kretschmann [8]. It uses a triangular prism on which the metal/dielectric interface is deposited. Various SPR biosensors based on this configuration were studied. Indeed, Baccar et al. [2] treated an SPR biosensor based on the Kretschmann configuration used for the detection of pathogenic bacteria (*Escherichia coli*). However, like any molecular analysis technique, this type of sensor suffers from certain limitations which can reduce their field of use and which are linked to the use of massive prism which has numerous disadvantages such as bulky size, complexity of assembly and high cost which makes it unsuitable for miniaturized applications and remote sensing. It should be noted that the use of this configuration is restricted to non-miniature systems. For this reason, many recent researches have focused on the development of new configurations to overcome these limitations and to realize efficient sensors. This has generated a recent interest for fiber optics as a new SPR sensor configuration. Numerous papers on fiber optic SPR biosensors have been published since 1992 [9]. As a result of this work, SPR fiber optic biosensors have been studied in all their aspects, such as the theoretical study of SPR fiber optic sensor with a bimetallic layer of Pt–ZnO [10]. In addition, the SPR sensor has been studied experimentally with different structures of the optical fiber [11]. In our previous study, the effect of graphene on the SPR response of a fiber optic biosensor has been investigated [12].

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In this study, an improved SPR biosensor with a high resolution is proposed. Compared with the old configuration, the present SPR biosensor is based on an optical fiber that takes the place of the prism, in order to simplify the detection process. A comparison of the response of two types of fiber optic SPR biosensors was performed. The first is metallized with a layer of gold. The second is based on a golden substrate functionalized with thiol acid. In addition, the detection of different concentrations of the *E. coli* bacterium was carried out.

To achieve this objective, the biological detection of bacteria by SPR has been studied theoretically. A numerical model based on a matrix formalism, which is initially developed by Abeles [13], has been then investigated. After that, we discuss the numerical results and especially the variation of the transmittance versus the wavelength.

2 Physical description: biodetection by SPR

The physical sensor has become a biosensor by immobilizing an active component (chemical or biological) on its surface. In order to study the biomolecular interactions by SPR, a receptor or "ligand" is immobilized on the metal surface and a target molecule or "analyte" constitutes the surrounding environment.

The resonance of the surface plasmons causes the metal reflection coefficient to fall, due to the energy adsorption peak of the incident beam near the resonance angle. The evanescent electromagnetic wave in the medium will be modified during the fixation of the molecules on the surface.

The SPR measurements are based on the refractive index, which makes it a quasi-universal detector. However, since the gold film is not selective by itself, it will be impossible using this configuration to distinguish a molecule in a complex mixture. Nevertheless, certain selectivity can be attributed to the gold film by chemically modifying the surface of the sensor. Indeed, it is possible to graft a molecular receptor selective to an analyte onto the gold surface, thus we can obtain an SPR sensor specific to a molecule, or a biomolecule in particular. In addition, upon injection of a solution containing the analyte of interest, the latter will be captured in solution by the molecular receptor placed on the surface of the sensor.

The choice of the sensitive layer is the basis of biosensor operation. The chosen metal layer will be in contact with the solutions containing the biological products involved in the measured interactions. The metal chosen must therefore be bicompatible: Do not react with the molecules involved in the analyzes and do not deteriorate over time following repeated contact with these liquid media (do not oxidize for example). This metal will also be the site of the functionalization of the surface and grafting of the probes and must therefore have physicochemical properties compatible with this need. The metal that meets all these criteria is gold. The elaboration of this sensitive layer is a critical step in biosensor design that is functionalized by a biological receptor layer on the gold surface. A literature review shows that usually the ligand is not directly attached to a gold surface. It appears necessary the presence of appropriate chemical groups to prepare the adhesion of the ligand. In addition the direct immobilization of the ligands on a gold surface could denature them. So, it would have a negative impact on the molecular interactions. It is therefore necessary to involve an important step in surface chemistry such as self-assembled monolayers (SAMs). This allows to deposit a biological layer on the gold surface via thiolized chains (thiol acid in this case). Consequently it is necessary to functionalize the gold layer with thiol acid.

The use of an SAM is a first option that will make it possible to functionalize the metal surface with the desired molecule, and then graft the molecular receptor onto the monolayer generated. The SAMs used in SPR are usually generated from bifunctional molecules and containing at least one thiol (SH) group. These SAMs made of thiolated organic molecules (11-mercaptoundecanoic acid (MUA)) generally form layers of about 1 to 3 nm thickness on the metal film [14] (Fig. 1).

The experimental set-up makes it possible to study the response of the sensor by varying the refractive index of the injected analyte. The fiber is cleaved at both ends and connected on one side to a white light source and on the other side to a spectrometer, after it is immersed in a cell or a series of *E. coli* solutions [15].

3 Numerical modeling: reflectivity and transmitted power

After applying a matrix calculation that was well adapted to the Matlab software and developed for the optical studies of multilayer media; and in particular for the optimization of optical fibers, this matrix method developed initially by Abeles [13] in the case of a multilayer structure plane, we have calculated from the Fresnel coefficients the reflectivity R_p of a plane polarized wave p on a medium consisting of N non-magnetic isotropic layers having dielectric constants ε_k. The electric field E_k and magnetic field H_k in the N layers are deduced using a transition matrix at each interface $(k-1)/k$ and a propagation matrix in each layer k. The first layer is the core of the fiber and the last one is the outer medium:

$$\begin{bmatrix} E_0 \\ H_0 \end{bmatrix} = M \begin{bmatrix} E_N \\ H_N \end{bmatrix} \tag{1}$$

This matrix M is thus the product of as many matrices as there are layers:

$$M = \prod_{k=2}^{N-1} M_k = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} \tag{2}$$

$$\text{with: } M_k = \begin{bmatrix} \cos\beta_k & \dfrac{-i\sin\beta_k}{q_k} \\ -iq_k\sin\beta_k & \cos\beta_k \end{bmatrix} \tag{3}$$

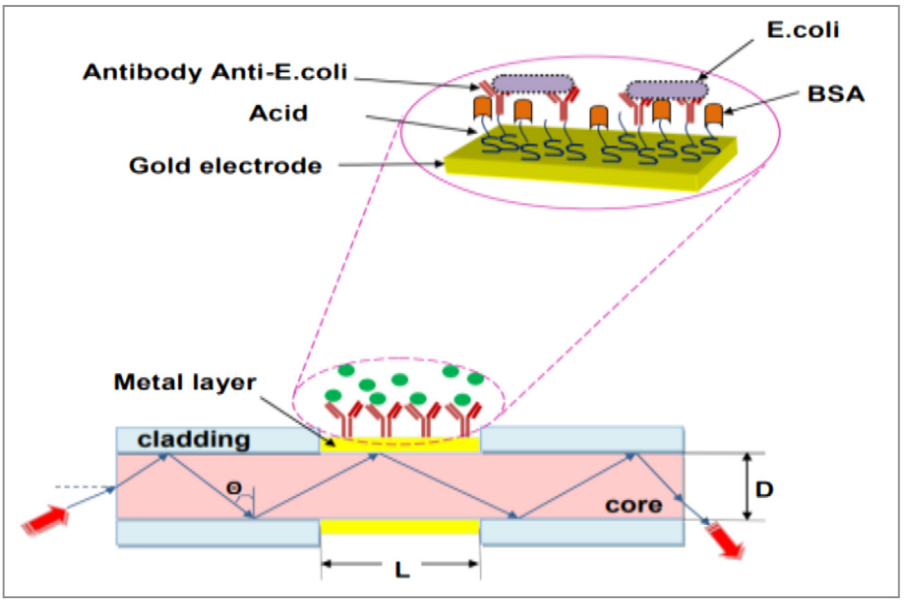

Fig. 1. SPR bacteria biosensor based on optical fiber.

The phase shift induced by each interface is defined:

$$\beta_k = \frac{2\pi d_k}{\lambda}\left(\varepsilon_k - n_c^2 \sin^2\theta_0\right)^{1/2} \quad (4)$$

The optical admittance is:

$$q_k = \frac{\left(\varepsilon_k - n_c^2 \sin^2\theta_0\right)^{1/2}}{\varepsilon_k} \quad (5)$$

The reflectance versus the wavelength is computed using the following equation:

$$R_p(\omega) = |r_p|^2 = \left|\frac{(M_{11} + M_{12}q_N)q_1 - (M_{21} + M_{22}q_N)}{(M_{11} + M_{12}q_N)q_1 + (M_{21} + M_{22}q_N)}\right|^2 \quad (6)$$

The transmitted power of each beam injected into the fiber depends on the reflectance R_p of reflected ray at the core/metal interface and the number of reflections $N_{ref}(\theta)$ [12] that will be subjected to the light ray characterized by its angle of incidence θ and its wavelength:

$$T = \frac{1}{2}\left(\frac{\int_{\theta_{cr}}^{\frac{\pi}{2}} R_p^{N_{ref}(\theta)} P(\theta) d\theta}{\int_{\theta_{cr}}^{\frac{\pi}{2}} P(\theta) d\theta} + 1\right) \quad (7)$$

The number of reflections is written as:

$$N_{ref}(\theta) = \frac{L}{D\tan\theta} \quad (8)$$

The power, dP, leaving the fiber between the angles θ_0 and $\theta_0 + d\theta_0$ [16] is proportional to:

$$dP \propto \frac{n_c^2 \sin\theta\cos\theta}{\left(1 - n_c^2\cos^2\theta\right)^2} d\theta \quad (9)$$

The critical angle is given by [12].

4 Results and discussion

A systematic experimental study on all the parameters could prove to be long and delicate, as the parameters are numerous. Therefore, in order to study and improve the performance of SPR biosensor, we have found that it is necessary to develop a numerical modeling tool. This model is based on the calculation of the reflectance as well as the transmittance as already mentioned. The geometrical configuration of the system studied is illustrated in the Figure 1. The effect of a variation of the analytical refractive index on the fiber optic SPR biosensor response for the detection of the pathogenic bacterium (*E. coli*) has been studied. The procedure described above makes it possible to obtain the spectral and the angular response of the biosensor.

4.1 Detection of *E. coli* bacteria by a gold-based optical fiber SPR biosensor

Figure 2 shows the numerical result of the spectral response of an SPR biosensor as a function of the wavelength for a series of solutions of the *E. coli* bacterium having different concentrations as shown in Table 1 (from 10^3 to 10^7cfu. ml^{-1}) corresponding to the various indices of refraction (from 1.3515 to 1.3547). These numerical simulations are carried out with a fiber optic biosensor of a sensitive length metalized with gold of 1 mm, a core diameter of the fiber of 200 µm, a deposit of gold of thickness 40 nm, and of an angle of incidence θ is equal to 82°. The curves in Figure 2 show the variation of the transmission as a function of the wavelength of the incident wave in the range 400–1500 nm.

It is clear that with the increase of the refractive index of sensitive medium, the peaks become increasingly wider by moving to higher resonance wavelengths (from 904 to 945 nm). The curves thus obtained reflect the excitation

Table 1. Variation in refractive index of *E. coli* layer as a function of *E. coli* concentrations [2].

E. coli concentrations (cfu. ml^{-1})	10^3	10^4	10^5	10^6	10^7
E. coli layer refractive index	1.3515	1.3530	1.3540	1.3545	1.3547

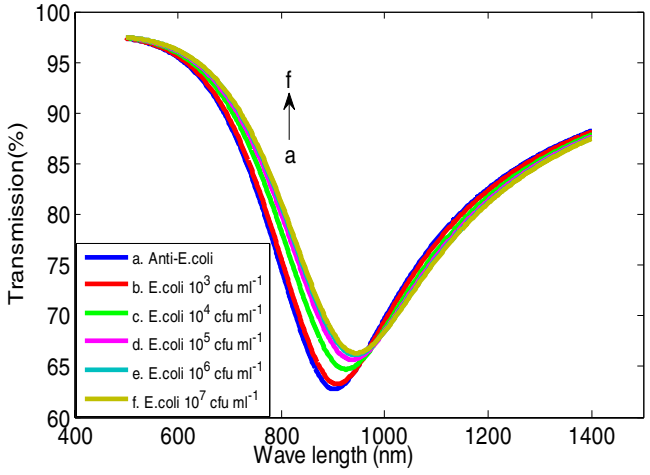

Fig. 2. The variation of the transmission of a metalized SPR biosensor with a gold layer as a function of wavelength for different concentrations of *E. coli*.

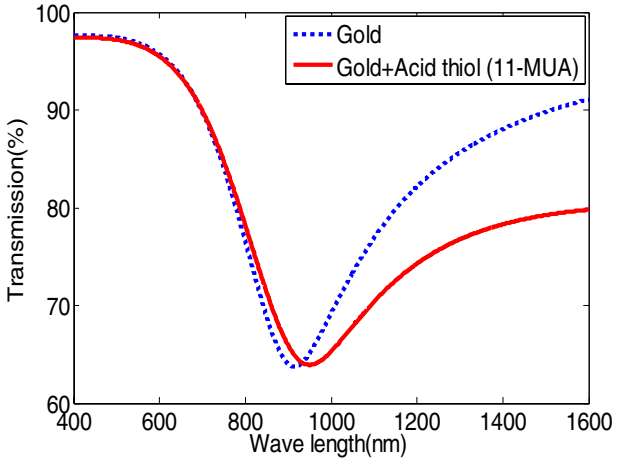

Fig. 3. The variation in transmission as a function of wavelength for gold and gold treated with thiol acid.

of the surface plasmon of the metal. We notice that a change in the concentration of sensitive medium results in a change in the index of the medium, which consequently causes a change in the shape of the resonance peak. This gives an indication of the evolution of the selectivity and the precision of the biosensor.

4.2 Deposition of thiol (the effect of thiol on the spectral response of the optical fiber SPR biosensor)

On the basis of the previous simulations, we will add another intermediate layer of thiol. (SAM of 11-MUA SAM). Its refractive index is $n(\lambda) = 1.433 + (1.589 \times 10^{-3\mu m2}) \times \lambda^{-2}$ [17]. We will therefore study the impact of this organic layer on the response of the biosensor. So, we will study the SPR response before and after the addition of thiol acid to assess the effect on the resonance wavelength λ_{res}.

Figure 3 illustrates the variation in the transmission as a function of wavelength of two biosensors which differ by the metal layer. The first metal layer is gold, while the second one is gold treated with thiol acid. The curves are obtained for a solution index of $n = 1.352$, a gold film thickness of 40 nm, a length of the sensitive zone of 1 mm, a fiber diameter of 200 µm, and a thickness of the thiol acid of 1.14 nm [2], for the second biosensor.

From this figure, it is clear that the resonance wavelength increases from 902 to 947 nm. This displacement is due to the absorption of the monolayer of thiol on the gold surface. The thiol group has a high affinity for gold and forms a quasi-covalent bond with this metal.

Due to the addition of the thiol acid layer, the resonance occurs with longer resonance wavelength and a higher sensitivity than without thiol acid. However, a slight broadening of the bandwidth was observed in the transmission spectrum. The first biosensor has the sharpest SPR resonance peak, but the second has much better characteristics in terms of sensitivity.

4.3 Detection of *E. coli* bacteria by a fiber optic SPR biosensor based on a golden substrate functionalized with thiol acid (gold with SAM)

As we have seen previously, an improvement in the SPR response was retained by treating the gold electrode with thiol. In order to evaluate the spectral response of a fiber optic SPR biosensor which is based on a gold substrate functionalized with thiol acid using the SAM technique, it seems interesting to study the behavior of this response to the different concentrations of *E. coli* bacteria.

Figure 4 shows the variation in transmission as a function of wavelength for different concentrations of *E. coli* bacteria ranging from 10^3 to 10^7cfu. ml^{-1}. The numerical results show that the resonant wavelength increases with increasing concentration. This is due to the specific recognition of the bacteria with the antibody. In fact, the increase in the concentration of the bacteria as shown in Table 1 induces an increase in the density of the layer, hence an increase in the refractive index which in turn causes a displacement of the transmission peak. Thus, when the refractive index of sensitive medium increases the bandwidth of the transmission spectrum becomes narrow with an increase in the resonance wavelength. This

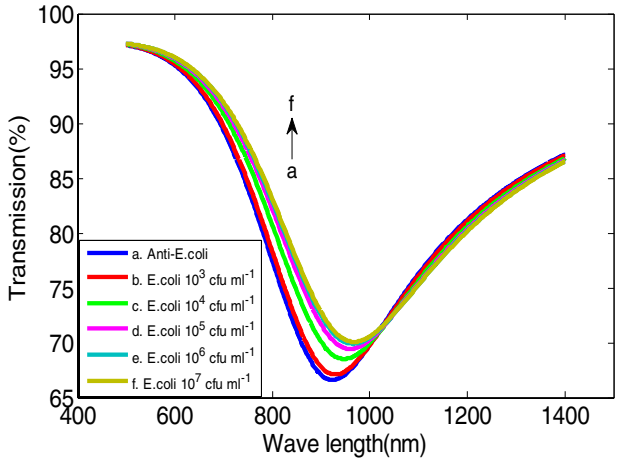

Fig. 4. The variation of the transmission of an optical fiber biosensor based on a gold substrate functionalized with thiol acid.

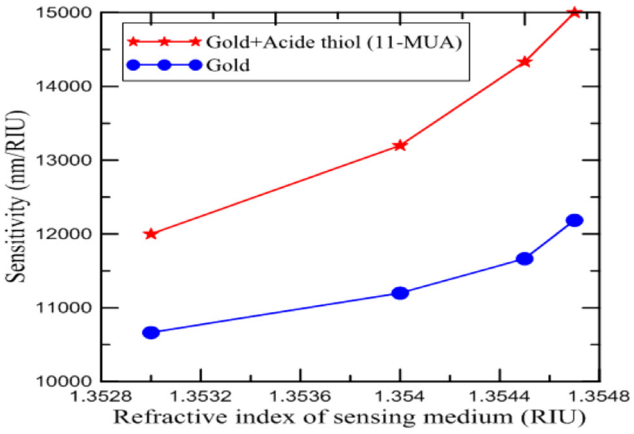

Fig. 5. Variation in the sensitivities of metalized sensors to gold and gold treated with thiol as a function of the refractive index of sensitive medium.

wavelength increases from 931 to 976 nm. A saturation of the signal was observed in Figure 4 in the range 10^5–10^7 cfu. ml^{-1}. A limit detection of 10^3cfu. ml^{-1} was obtained with good reproducibility.

The results of the present work are comparable to those presented by Baccar et al. [2] on immuno-sensors based on SPR for the detection of bacteria. They have studied the angular response of two biosensors based on the conventional prism configuration. The first was based on a golden substrate functionalized with thiol acid using the SAM technique, while the second one was functionalized with gold nanoparticles immobilized on modified gold. From a complete experimental study, they show that the sensitivity of these biosensors increases by immobilizing gold nanoparticles on golden substrates. Indeed, the limit of detection for the first biosensor is 10^4cfu. ml^{-1} whereas for the second is 10^3cfu. ml^{-1}. From this work, they were able to develop an immuno biosensors for the rapid detection of two bacteria (*E. coli*) and (lactobacillus).

Similarly to what we have obtained in the comparison between the spectral response of the biosensor based on gold substrates and that of the biosensor based on gold substrates treated with thiol acid (Fig. 3), Baccar et al. have found the same behavior of the reflectivity curves as a function of the angle of incidence. An angular displacement of 0.54° was recorded by Baccar et al. For the detection of *E. coli* bacteria using gold with SAM (Fig. 4), we can note that the numerical response of the biosensor for a concentration ranges of 10^3 to 10^7cfu. ml^{-1}. This result is comparable to the experimental results presented in the works of the other authors [2,18].

4.4 Comparison of the sensitivities of the metalized sensors to gold and gold treated with thiol

The sensitivity $S_{\lambda n}$ of the sensor is defined as the ratio between the variation of the parameter characterizing the plasmon resonance, in this case the wavelength λ_{res} for which the transmission through the fiber is minimal, and the quantity to be measured (the refractive index in this case). Thus, the sensitivity of the sensor will be equal to [19]

$$S_{\lambda n} = \frac{\delta\lambda_{res}}{\delta n_s} (\text{nm RIU}^{-1}) \quad (10)$$

To better justify the importance of the addition of the organic layer (thiol acid) to the sensitive gold layer on the SPR response of the biosensor and on the sensitivity, Figure 5 represents a comparison between the sensitivities of the metalized sensors to gold and gold treated with thiol. The two biosensors having the same characteristics mentioned above.

It can be observed that, the two curves follow the same evolution when the refractive index increases. Indeed, the curve in blue increases until reaching a maximum value of sensitivity equal to 12 187 nm RIU^{-1}. The red curve also increases to a maximum of 15 000 nm RIU^{-1}. It is clearly remarkable that the sensitivities obtained on gold-based fibers treated with thiol are always more favorable than those obtained on the gold-based sensors. As a result, the addition of thiol acid has a significant effect on the sensitivity of a gold-based biosensor.

4.5 Influence of thiol layer thickness on SPR response

Figure 6 describes the variation of the transmission curve as a function of wavelength for different values of the thickness of the layer of the thiol acid. The refractive index of sensitive medium is 1.352. The geometric parameters of the fiber are kept constant. According to this figure, the general shape of the curve does not vary. However, the variation of the resonance wavelength is inversely proportional to the variation of the thickness. When the thickness of the thiol acid layer increases, the resonance wavelength decreases. It varies from 939 nm for $d = 1$ nm to 898 nm for $d = 3$ nm. The figure also shows a slight variation of the transmission spectrum bandwidth by changing the thickness from 1 to 3 nm.

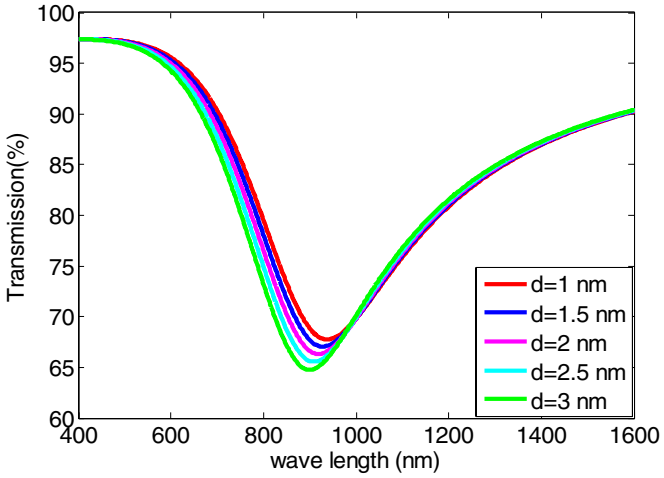

Fig. 6. Influence of thiol layer thickness on SPR response.

5 Conclusion

This article is a numerical study of a new configuration of SPR biosensor, applied in the detection of pathogenic bacteria (*E. coli*). This biosensor is based on the optical fiber that replaces the conventional prismatic configuration. From this numerical study, we have been able to show that the numerical model implemented is relevant, and can be used to study the response of a biosensor instead of conducting experiments.

The spectral study carried out in this article clearly demonstrates that the bio functionalization is a key step for obtaining good analytical performances. The sensitivity and the selectivity of this biosensor are improved thanks to the anti-body (anti-*E. coli*) attached on the thiol SAM layer which contributes indirectly in the enhancement of this sensitivity.

Author contribution statement

Yosra Saad wrote the manuscript text, performed the calculation and prepared the figures. Marwa Selmi and Mohamed Hichem Gazzah evaluated the results and contributed to the numerical simulation. Hafedh Belmabrouk checked the final version of the manuscript and contributed to the interpretation of the figures.

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