Fibre-Optic Microsensor Based on Surface Plasmon Resonance in a Microfluidic Cell : an Experimental and Numerical Multiphysics Approach

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Abstract: In the last decade, the surface plasmon resonance (SPR) has become a very sensitive technique for real-time detection in many application areas. Considering the important needs for analyzing biomolecular reactions through automated and miniaturized components, fiber optic sensors based on the SPR technique are becoming an important choice in the development of sensors. In the present work, a microfluidic system associated with a fiber optic SPR sensor is developed to monitor in real-time the variation of sensor's responses to each kinetic reaction occurring at the surface. Using kinetic parameters, produced by our experimental measurements, in our numerical model enables us to demonstrate the potential of fiber optic SPR sensor for biological analysis purposes.

Keywords: SPR, surface plasmon, fiber optic sensor, microfluidic cell.

1. Introduction

Today, many microbiological analysis laboratories use "chips" or microsystems. The main reason for using these systems is their automation and miniaturization, making possible an integration of the measurement operations to only one device while visualizing and handling objects as soon as possible. In biotechnology these objects are often alive targets. In the majority of the cases, in order to not kill the studied targets, it is important to be able to handle them in small volumes of fluids which circulate in channels having micrometric or nanometric dimensions. This handling of the fluids is known under the term of "microfluidic".

Due to the significant improvements in biotechnology, the microfluidic has become a separate branch in the mechanics of fluids, and the technologies associated with manufacturing the substrates which are known as "lab on chip" are progressing rapidly. This progress is because of the strong demand for handling the samples of very small sizes and very small quantities, as well as the interest to increase the ratio "surfaces on volume" during surface chemical reactions, which is a good case for SPR studies. Thus, together with the biosensors, the microfluidic devices make it possible to automate and increase the flows to carry out complex screening on cellular or molecular entities [1-4].

In this work, our objective is to optimize the performance of a fiber optic sensor based on surface Plasmon resonance (SPR). To achieve this goal, it is necessary to develop a numerical model which enables us to determine the optimal operating conditions of the sensor by carrying out numerical simulations using certain parameters, resulting from the experimental data. This led us to study and model (I) the flow and the transport of mass by diffusion and advection of target molecules inside a microfluidic cell; (II) the reactions of hybridization of target molecules with ligands; (III) the sensitivity limits of such a system.

2. Results and modeling

2.1 Modeling of the fiber optic index sensor

The adsorption of target particles on the surface results in a variation of the optical signal transmitted in the fiber optic by SPR effect [3]. At the same time, a gradient of concentration is created in an area known as "limiting area of concentration" on the fiber surface, increasing the phenomena of diffusion, and modulated by the supply of solution. This will result in the fast establishment of a balance of concentration on the surface of target molecules visualized by the curves called "sensorgrams" (see Figure 1, above side).

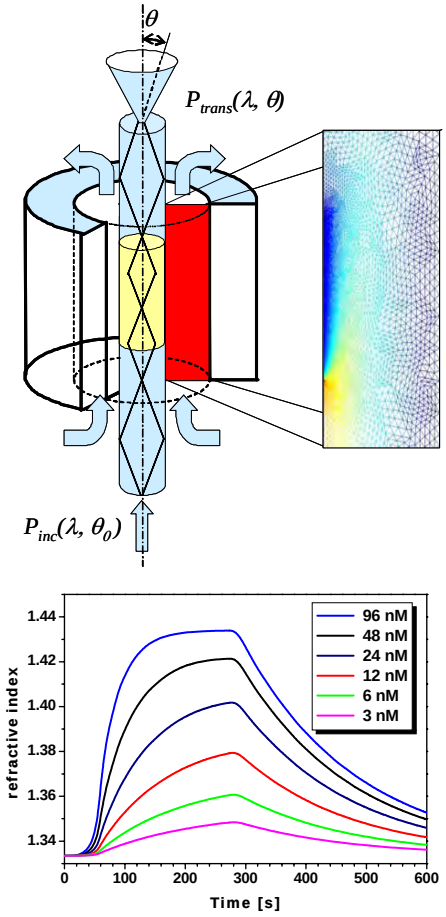

Figure 1. Principle of the measurement by fiber optic SPR sensor.

The biological targets were diluted in a liquid (buffer solution) in order to be guided towards a reactive surface where they are immobilized during the time of measurement. Then, various forces operate on these targets. In a microscopic scale, we should take into account the diffusion, representing the random displacement of particles of the Brownian movement, the phenomena of advection (forced convection) of the particles in the moving fluid, and the mechanisms of hybridization (i.e. adsorption and desorption) of the particles on the surface of the sensor.

The reactions of surface, in particular the phenomena of adsorption and desorption, are then taken into account by using the model of Langmuir, implemented for the calculation of the concentration on the surface of the analyte. These results are used for the calculation of the transmission of the light in the optical fiber from the Fresnel equations, and Kretschmann-Raether model [3,4].

In order to model the experiments, we have to couple with the calculation of the optical reflectivity, and the effects of material transport. The equations used to solve the flows in a microfluidic system generally derive from the Navier-Stokes equations and are associated with simplifying assumptions, particularly about the geometry of the channels.

In our cell, the channels have a dimension of approximately 300 µm. We worked with speeds of the fluid about 1 to 50 mm/sec. At these scales, turbulence is absent in our microfluidic system, and the flows to be modeled are laminar, and the fluids are incompressible, homogeneous and Newtonian [6].

For the resolution of the partial derivative equations (PDEs) and for the discretization of space, the software Comsol Multiphysics 3.5 was used. The coupling with the optical measurements was implemented under Matlab 7. Figure 2 depicts the experimental and simulation curves of the light transmission at 600 nm according to the time passed starting from the injection of the glycerol solution in the cell.

The experimental results are compared with the numerical results in order to validate the simplifying assumptions which we introduced at the time of the modeling of the phenomena of flow. The optical effects induced by the plasmon resonance of SPR surface and the partial derivative equations representing transport of the analytes were calculated simultaneously while taking into account the geometry of the cell.

2.2 Reaction of bio-recognition and model of Langmuir

Basically, the objective of a SPR sensor, and more generally of a biochip, is the analysis and recognition of target macromolecules (DNA, proteins, etc). The bio-recognition is based on a mechanism called key-lock. We can define a biomolecular reaction as the association of two molecules to form a “complex”, for example two complementary monobrins of DNA. Among the various models of heterogeneous molecular reactions, we chose the model of Langmuir.

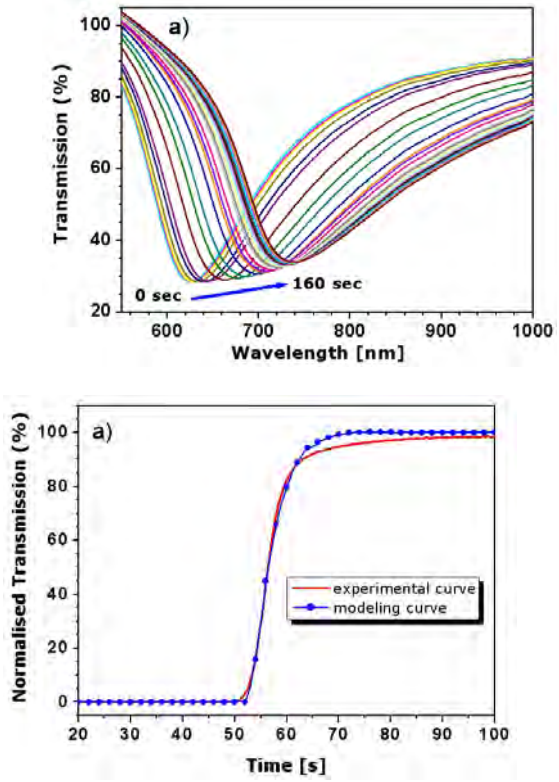

Figure 2. Principle of the measurement by fiber optic SPR sensor a) spectral response of fiber optic SPR sensor during the injection of a glycerol/water solution (n_S = 1,3820); b) optical response measured at 600 nm (U = 10 mm/s ; D=10-9 m^2/s ; C_0 = 5,43M).

This model uses the concepts of association phenomena and/or dissociation which determine the affinity of a ligand (L) for an analyte (A).

In a microfluidic cell, the analyte is transported towards the surface by convection and diffusion because it is injected in continuous flow. The ligand-analyte reaction, particularly its kinetics, does not depend only on the reaction speeds but on the processes of mass transport close to the surface. The rate of coating is calculated by the resolution of the following equation 1:

$$\frac{d\Gamma}{dt} = k_{ass} c_{surf} (\Gamma_0 - \Gamma) - K_{diss}\Gamma$$

We see here the appearance of the coupling between the reaction of the surface and the mechanisms of transport via the calculation of the concentration csurf , where csurf is the concentration of the analyte close to the surface.

To determine the concentration csurf of the occupied or hybridized spots on the surface, we have to introduce the balance calculation of the masses on the surface, taking into account the diffusion on the surface and the reaction speed expressing the formation of adsorbed species:

$$\frac{\partial c_{surf}}{\partial t} + \nabla \cdot \left(-D_s \nabla c_{surf}\right) = \frac{d\Gamma}{dt}$$

With

$$\Gamma = \frac{D\nabla c_{surf} + k_{ass} c_{surf} \Gamma_0}{k_{ass} c_{surf} + k_{diss}}$$

2.3 Kinetic study of the PKI-PrKX reaction

The consideration of the ligand-analyte interaction (formation of the organic layer) in our numerical model requires introducing the values of the kinetic parameters (kass et kdiss) of the molecular reaction to describe the boundary condition of the surface of the sensible area. For this, we used the experimental results obtained by B. Zimmermann et al. [7].

Figure 3 presents the experimental sensograms and modeling of the PKI protein (protein kinase inhibitor) that plays the role of the ligand immobilized on the surface of a biosensor and PrKX protein (human X chromosome protein kinase) that plays the role of analyte. The values of kass and kdiss obtained after the processing of the signal by the software Bia-evaluation are respectively 3.8 10^5 $M^{-1}s^{-1}$ and 5.8 10^{-3} s^{-1}.

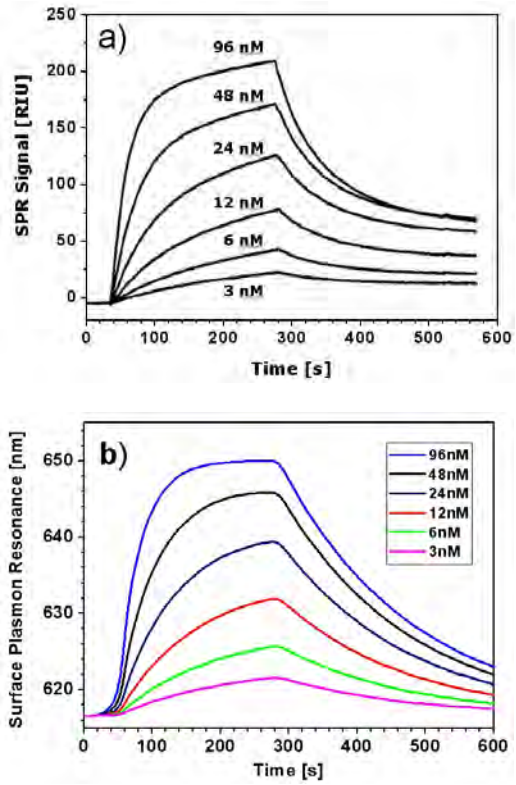

Figure 2. a) SPR response measured at the time of the molecular interaction for the protein PKI (Ligand) and the protein Kinase X (analyte) according to Zimmermann and al. [7]). b): Variation of the index of the biological layer according to time for various values of concentration of the analyte (U=6mm/s ; D = 10^{-10} m^2/s ; kass= 3.8 10^5 $M^{-1}s^{-1}$ and kdiss =5.8 10^{-5} s^{-1}).

By this study we found the means of validating our numerical model, and numerically studying the response of fiber optic SPR sensor.

From these curves we can extract a sensitivity of our sensor of 0.6 nM/nm and mass sensitivity of our sensor of approximately 12ng/nm (equivalent mass for a biological layer thickness of 20 nm)

3. Conclusions

In this work, we studied the response of a fiber optic SPR sensor SPR in the presence of an analyte subjected to a laminar flow. This flow was carried out using a microfluidic cell in which the fiber sensor is placed. The goal of this study was to evaluate the effectiveness and the sensitivity of this sensor in a real operational configuration.

For a model of molecular interaction of PKI protein and PrKX protein, we could determine a mass sensitivity and concentration of about 12 ng/nm and 0.6 nM/nm respectively. Notice that that these results are valid for molecules of important size leading to a biological layer on the surface with a thickness of 20 nm. In conclusion, in this work it is shown that a fiber optic SPR sensor, associated with a microfluidic cell, is capable to carry out biological analyses, whether they are quantitative or qualitative.

8. References

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