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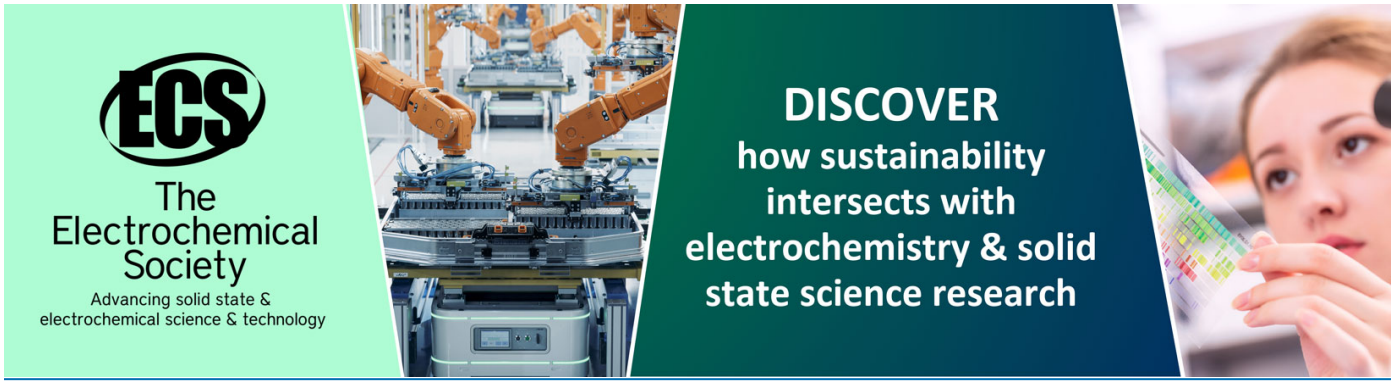

Development of an Escherichia coli optical biosensor with computational validation

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Abstract. The biosensor consists of an optical sensing system and an optoelectronic data acquisition system. The sensor's optical system consists of a biochemically functionalized polymer optical fiber (POF-Plastic Optical Fiber) based on Field Evanescent technology. The Evanescent Field technique has been widely adopted in sensing and in this project, it was obtained by bending the fiber in a "U" shape, aiming to increase the sensitivity of the biosensor, through the contact of the curved sensor part with the sample biological. A data acquisition system was developed through an optoelectronic project aiming to increase the sensitivity when compared to a commercial equipment acquisition system. This work presents a biosensor for the detection of Escherichia coli based on an evanescent field with a polymer optical fiber linked to the analog signal acquisition system through an optoelectronic system developed. The interaction investigation of antibodies and antigens in Escherichia coli for computational methods was carried out in order to obtain information about the action of the antibody and in future steps applied in the validation of the diagnostic method.

1. Introduction

There are several methods for the identification of bacterial agents, the most used being bacteriological culture. This methodology has a high cost because it requires time, professionals, and qualified laboratories.

The development of biosensors for the detection of biological and chemical analytes is a great help for characterizations of this type in the laboratory and clinical environments [1].

Currently, Foodborne Diseases are a public health responsibility, as they cause hospitalizations due to food contamination by bacteria [2]. The most common bacteria that cause Foodborne Diseases are species of the Escherichia coli group.

This work presents the development of an Escherichia coli biosensor that consists of an optical sensing system and an optoelectronic data acquisition system. The sensor's optical system consists of a biochemically functionalized polymer optical fiber (POF) based on the Evanescent Field effect. The Evanescent Field technique has been widely adopted in several works, including previous ones from our group [3].

In recent years, the detailed structures of a number of antibody/protein antigen complexes have been reported, providing some insights into the mechanism and the specificity of the interactions [4]. The interaction investigation of antibodies and antigens in Escherichia coli for computational methods was carried out in order to obtain information about the action of the antibody and in future steps applied in validation of the diagnostic method.

2. Materials and Methods

2.1. Escherichia coli biosensor

The biosensor is divided into two parts: an optical sensing system and an optoelectronic data acquisition system. The first refers to the optical system of the sensor through a polymer optical fiber (POF-Plastic Optical Fiber) biochemically functionalized and based on Evanescent Field technology. The Evanescent Field technique was obtained by bending the fiber in a "U" shape, aiming to increase the sensitivity of the biosensor, through the contact of the curved sensor part with the biological sample. The guidance of light in the POF comes from an LED (Light Emitter Diode), properly coupled to one end of the fiber, and a photodetector, responsible for the optical to electronic conversion, positioned at its other end. The second stage consisted of the development of an optoelectronic data acquisition system. Figure 1 shows the complete biosensor system.

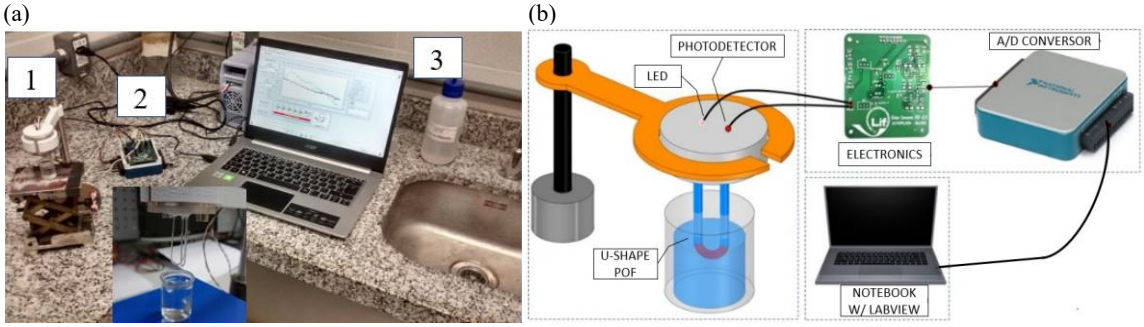


Figure 1 - The complete biosensor system. (a) Photo of the complete biosensor system: 1-Sensing system, 2-Electronic system and 3-Notebook. (b) Schematic diagram of the complete biosensor system.

Figure 1 (a) shows the optical sensing system: (1) composed of a U-Shaped POF fiber (1 mm in diameter and with a Polymethylmethacrylate-PMMA core, Mitsubishi Rayon Company), an LED (SFH 485 P), and a photodetector (SFH 203 P) and the electronic system. (2) which consists of a developed electronic circuit, a signal acquisition board (DAQ model USB-6001, National Instruments), software developed in the NI LabVIEW program (DAQPO_vF3.2 program), and notebook (3). Figure 1 (b) shows the schematic diagram of the complete biosensor system. An electronic circuit was developed responsible for driving the LED, amplifying the signals generated by the photodetectors, and sending these signals to the acquisition board, DAQ. The DAQ board has the function of converting the analog signals generated by the electronic board into a digital signal, and sending the data obtained to the notebook. Finally, the notebook presents these digital signals in the form of a graph, through the LabVIEW program, in which we monitor all the information provided by this electronic board. The basic operating principle of the biosensor consists of detecting the refractive index (RI) of the biological

material to be tested and, through the electronic system, correlates the refractive index with an electronic signal generated in a fast response time. The following steps present the development of the biosensor:
1 - POF sensor fabrication: a POF of 1000µm (Multi-mode Mitsubishi Rayon Eska GH 4001) was placed in a "U" shaped mold and then placed in the oven at a temperature of 70°C for a period of 60 minutes;
2 - Functionalization in POF fibers: first, the Escherichia coli antibody (Polyclonal anti-E. coli antibody from rabbits, Bio-Rad Labs) is immobilized on the fiber surface using protein A. After this step, functionalization with the PEI (Polyethyleneimine) is responsible for the release of amines (reactive and biocompatible chemical groups) for antibody fixation;
3 - Afterwards, the tests are performed: Initially, we access the software (DAQPOF_v3.3) and choose a certain electrical voltage value for the LED (2V - 2.5V);
4 - POF fibers in the support: place two fibers in the support, one fiber without functionalization, as a reference and another functionalized, as a sensor;
5 - Calibration: the calibration is performed using the refractive index of air as a reference;
6 - Subsequently, the fibers are placed in a beaker with Escherichia coli bacteria (O55, Oswaldo Cruz Institute, Rio de Janeiro, Brazil). The fibers are in contact with the Escherichia coli sample for around 10 minutes;
7 - After this period, the fibers are removed from the beaker and washed with ultrapure water, to remove all bacteria that are only on the surface and are not chemically linked to the antibody;
8 - Calibration: we start the calibration again using the air refractive index as a reference and the functionalized fiber is placed in contact with the Escherichia coli solution in the beaker;
9 - Finally, the principle of operation of the biosensor occurs through the increase of the electrical voltage when the functionalized POF is in contact with Escherichia coli when compared to the voltage value during the calibration process with air. This process takes about 2 minutes.

2.2. Investigation of interactions by computational methods

To obtain insights into the role of the immune system on E. coli antigens, a search was performed on the Protei Data Bank (PDB) database. The tertiary structure of Jel42 Fab fragment complexed with HPr (PDBid: 2JEL) was selected to carry out the computational investigation of these interactions. This antigen is a phosphocarrier protein of the phosphoenolpyruvate: sugar phosphotransferase system of Escherichia coli, and has been determined by X-ray diffraction at 2.5 Å resolution [5]. The visualization of the structures and the analysis of the most representative residues in the antibody-antigen complex were performed using the Pymol software.

Finally, the antigen/antibody evaluation study is being carried out in order to qualitatively validate the interaction of the complex, confirming its compatibility. The K antigen lead epitopes will be evaluated for interactions with the antibody CDR receptor, using the SwissDock server (http://www.swissdock.ch) for this purpose. The interactions will be evaluated for electrostatic surface, contact interface and hydrophobicity, using programs such as Pymol and Maestro to select the best complex.

3. Results

The experimental tests performed validated the adopted techniques and the partial results obtained are presented below.

3.1. POF sensor response

Figure 2 shows the POF sensor response under the variation of the refractive index, measured in sucrose solutions, during all the steps of the functionalization process with PEI.

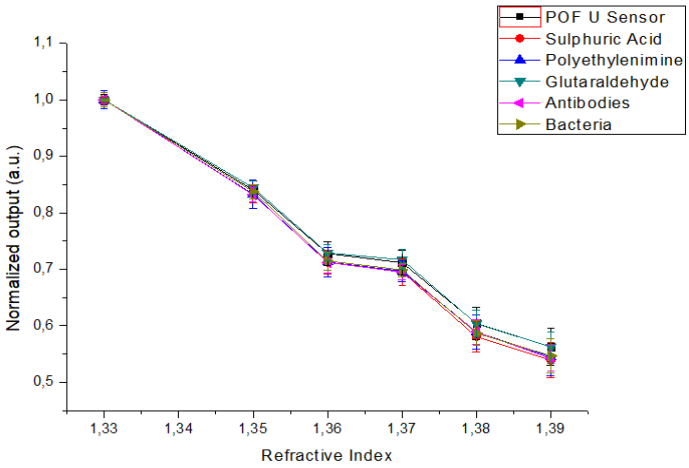
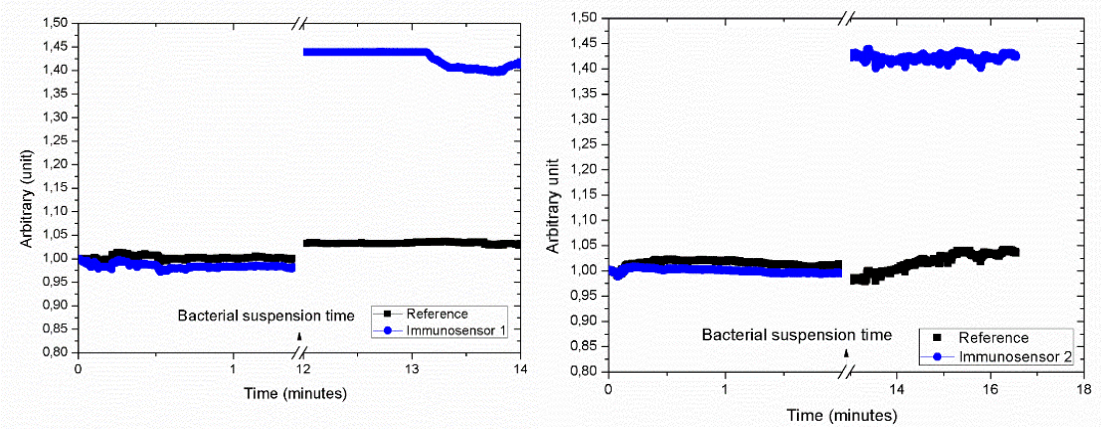


Figure 2 - Response of the "U" shaped POF sensor under the variation of the refractive index, measured in sucrose solutions, during all the steps of the functionalization process with PEI.

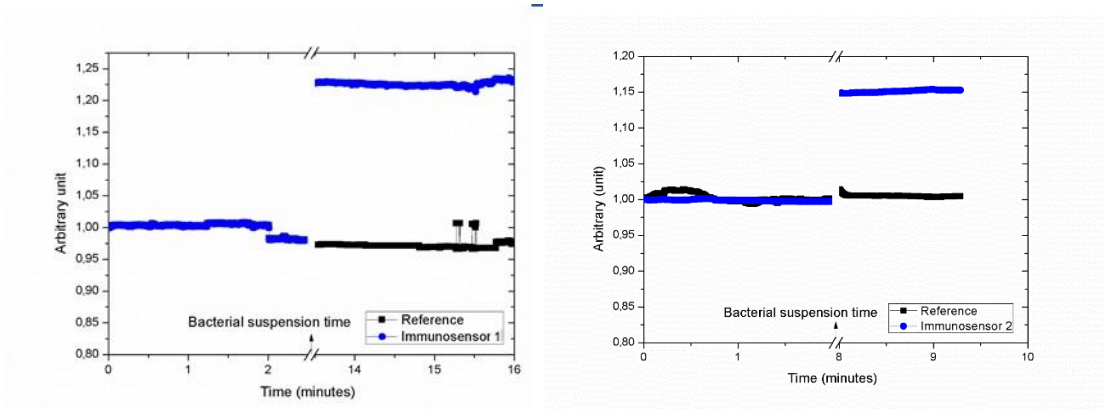
It was observed that there was no deterioration or loss of sensor sensitivity after the chemical and biological processes of the functionalization process. It was also observed low dispersion, after the immobilization of the antibodies. After all the functionalization processes and the refractive index measurements, the biosensors were subjected to the detection of *Escherichia coli*. The biosensors were able to detect the presence of the bacterium *E. coli* in the suspension of 10^8 CFU/mL as illustrated in Figure 3.



(a) Biosensor 1 (b) Biosensor 2

Figure 3: Biosensors in the detection of Escherichia coli in the bacterial suspension of concentration 10^8 CFU/mL.

Lower concentrations of solutions with Escherichia coli bacteria were also tested and the system was able to detect the presence of the bacteria with concentrations of 10^4 CFU/mL. Figure 4 shows the result of biosensors with bacterial suspension of 10^4 CFU/mL of *Escherichia coli*.



(a) Biosensor 1 (b) Biosensor 2

Figure 4: Biosensors in the detection of E. coli in the bacterial suspension of concentration 10^4 CFU/mL

It can be seen that the biosensor developed was able to detect bacterial concentrations of 10^8 CFU/mL and 10^4 CFU/mL, validating the technologies adopted for the development of the biosensor. Lower concentrations could not be detected due to the limitation of the antibody which is 10^4 CFU/mL.

3.2. Computational preliminary investigation

The selected structure Jel42 Fab/HPr represent a complex, the antigen fits into a distinct depression in the binding site. Other protein-binding antibodies tend to have a flatter binding surface. The buried surface area of the interface is approximately 700 Å2, which is typical of most antibody/antigen complexes (Figure 5).

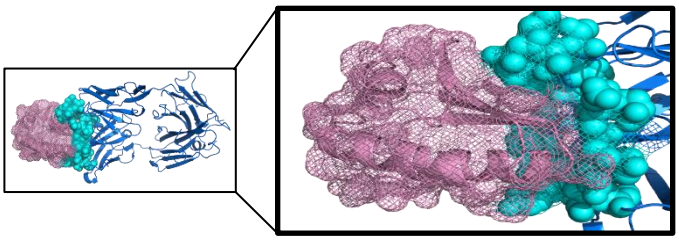


Figure 5: Representation of the electrostatic surface of the complex formed by the antibody (blue marine) and antigen fragment protein (grape) Jel42 Fab/HPr complex. In magnification (cyan) the region of contact with the light chain of the antibody, interface is approximately 700 Å2. Representation of the structure obtained in the Pymol software.

The binding surfaces of the antibody and antigen are generally sterically and electrostatically complementary. Polar regions are preferred for the epitope profile, to represent a region with a more hydrophobic profile and favorable to interaction [4] (Figure 6).

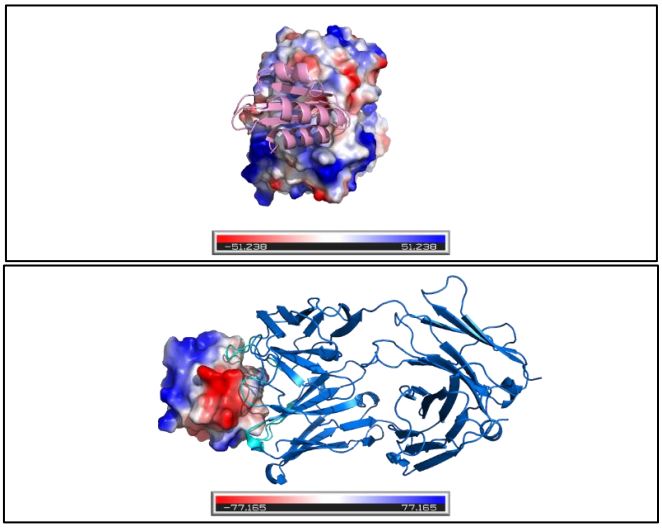


Figure 6: Representation of the electrostatic surface of the complex formed by Jel42 Fab/HPr. A) The region of antibody interaction - on surface mode representation - with the epitope contact region, reveals a neutral charge, represented by white, and positive, in blue. B) It is possible to see that most of the surface of the antigen (represented in surface mode) in contact with the light chain of the antibody (orange) is negatively charged, colored in red. Representation of the structure obtained in the Pymol software.

According to the results, the contact interaction region located has a polar profile, therefore, is more hydrophilic. In return, the contact region of the antibody light chain has a profile ranging from neutral to nonpolar, and therefore more hydrophobic, with the interaction with the predicted epitope being favorable. The regions of interest that will be investigated in the antigens must have the characteristics visualized in the complex above. In this way, this representation helped in making decisions regarding the filters that will be applied in the following predictions.

4. Conclusions

It is concluded that the biosensor was able to detect concentrations of 10^8 CFU/mL and 10^4 CFU/mL of *Escherichia coli* with the optoelectronic data acquisition system developed. With the validation of the system proposed here, we hope to test the biosensor with other emerging pathogens specific to the State of Rio de Janeiro, Brazil. The computational investigation is preliminary but indicates that the antigenic regions to be used in the development of the biosensor must have the known characteristics, as evidenced here. Previous computer analyzes were crucial to follow up on the following steps, which are already in progress.

5. References

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