Protein detection by polymer optical fibers sensitized with overlayers of block or random copolymers

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ABSTRACT

In this study a low cost and low complexity optical detection method of proteins is presented by employing a detection scheme based on electrostatic interactions, and implemented by sensitization of a polymer optical fiber (POF) surface by thin overlayer of properly designed sensitive copolymer materials with predesigned charges. This method enables the fast detection of proteins having opposite charge to the overlayer, and also the effective discrimination of differently charged proteins like lysozyme (LYS) and bovine serum albumin (BSA). More specifically, as sensitive materials here was used the block and the random copolymers of the same monomers, namely the block copolymer poly(styrene-b-2-vinylpyridine) (PS-b-P2VP) and the corresponding random polymer poly(styrene-r-2-vinylpyridine) (PS-r-P2VP), of similar composition and roughly similar molecular weight. Moreover, this work focused on the comparison of the aforementioned sensitive materials regarding the way in which they can adapt on sensing optical platforms and constitute functional sensing bio-materials.

Keywords: Block copolymers, Random copolymers, Optical Biosensors, Proteins, Fiber sensors

1. INTRODUCTION

Over the past decades a remarkable progress has been observed in bio-molecular research, largely stimulated by the flourishing field of materials science and particularly from the development of new polymeric materials. The selection, characterization and composition of functional materials suitable for bio-sensing applications is urgently required thus several studies have been reported concerning the differentiations on morphology, chemical synthesis and physicochemical properties of block and random polymers[1-3]. A major sector of bioassays associated with the ability to trace biomolecules such as proteins, since proteins play a key role in cellular processes and diseases diagnosis, making the need of detection very important, in biological and biochemical research, biotechnology, food analysis and clinical diagnostics[4]. So far protein detection is based mostly on expensive and complex spectroscopic techniques, such as surface plasmon resonance (SPR)[5] for label-free detection, fluorescence detection which enables the identification of specific protein modifications, nanoscale biosensors that use aptamers as molecular recognition[6] and sensitive surface-enhanced Raman scattering (SERS)-based biosensors using optical fibers for label-free macromolecule detections[7].

Although these detection techniques provide highly sensitive detection (< 0.2 μg/mL) of a target analyte, there is a need for rapid, simple and low cost detection method adaptable to functional platforms. Several studies have proved the functionality of the POF platform for bio-detection, while the increase of the sensitivity of this type of sensors is still under study. Recently De Nazare et al. (2011) evaluated a series of optical fiber taper sensors to achieve the best tapering characteristics which will provide an increased sensitivity[8]. Beres et al. (2011) use U-shaped chemically treated POF with immobilized antibodies to detect target cells, indicating the POF biosensor as a potential device to detect cells in aqueous medium.

In this work we follow an alternative approach regarding the protein detection without using recognition elements, as already mentioned to our previous study[9]. The efficiency of this method relies, firstly, on the absorption of the proteins from specific sensing materials and secondly on the interaction of the increased evanescent field (EF) with the sensing materials. Parameters such as the adsorption of proteins from the active materials and the chemical modification of the polymer substrate surface are very important during the detection process, thus many investigations are devoted to studying the adsorption mechanism of proteins from multi-component systems on different surfaces[10] and the procedure of proper chemical treatment of such polymer surfaces[11].

2. EXPERIMENTAL

2.1 Materials

HCl (Alsdrich) was diluted in deiionied water in order to prepare a 1M solution. PS-b-P2VP block copolymer was synthesized by anionic polymerization[12-13], while the corresponding random copolymer Ps-r-P2VP was synthesized by radical polymerization using 2-vinylpyridine and styrene as the monomers and AIBN as the polymerization initiator in dioxane[13]. In table 1 the molecular characteristics of the copolymers used are shown.

Table 1. Molecular characteristics of the copolymers used.

Sample	M_w x 10^3 (by SEC/NMR)	M_w/M_n (by SEC)	Composition (by ^{1}H NMR)
PS-b-P2VP	7.04	1.01	44 wt % PS
PS-r-P2VP	4.53	2.14	47 wt % PS

Both block copolymer PS-b-P2VP and random copolymer PS-r-P2VP were dissolved in THF in order to prepare polymer solutions of concentration ca. 50mg/mL.

2.2 Sensors' Development

The functionalization of the polymer fiber active region is achieved, firstly, by removing the jacket and the fluorinated polymer cladding (Fig. 1a), thus exposing the fiber core as sensing zone, followed by proper chemical treatment of the PMMA surface, which generates an area with improved bio-contact properties and, in parallel, gives some additional properties that influence the procedure in which sensing materials are coated. The optical fiber was permanently bended, with the angle of curvature approximately 180◦ (Fig. 1b), in order to enhance the penetration depth of evanescent wave and hence the sensitivity of the probe. This procedure was conducted by using a heat gun, which exhibited a bend loss of around 3 dB, while the fluorinated polymer cladding of a 5cm effective probe length was removed with 30% solution of acetone in deionized water. The chemical modification of the exposed active fiber region allowed us to intervene on the fiber surface topography thus influencing the wettability and hence the protein adsorption.

More particularly, two methods were followed. In the first method, the change of the hydrophobic nature and the positive charge of the PMMA surface is achieved by immersing the active region of the fiber in isopropanol and sodium hydroxide 0.1 M respectively, while in the second method, in order to further increase the hydrophobicity of the PMMA surface, the fiber was immersed in 0.1 M cyclohexane. The aforementioned methods were evaluated concerning the final responsivity of the sensor. Subsequently, proteins were physically adsorbed in different ways from the sensing materials which were successfully coated on modified fiber surfaces, as it was proved with IR spectroscopy (Fig. 2). Comparing the ATR-FTIR spectra of the sensor probe before and after the deposition of the sensing material, it was observed that intensity peaks corresponding to the styrene/2-vinylpyridine copolymers immerged while the main peaks of PMMA at 1157, 1398 and 885 cm^{-1} are essentially absent. This result indicated the presence of copolymer coating layer on the PMMA fiber surface and proved the efficient deposition of the sensing material on the PMMA fiber surface. The deposition of polymeric thin films onto PMMA fiber tips was achieved by using the commonly used dip coating technique, which allowed the quick (within 6 min) and stable (over a month) formation of a layer using low-complexity and inexpensive infrastructure. After the deposition of the sensing material, the active coated region of the fiber was immersed in HCl 1 M and washed with dionized water to redistribute the blocks/segments of the copolymer (PS-b_P2VP or PS-r-P2VP) in order the protonated (positively charged) 2VP segments to be transferred to the outer material surface.

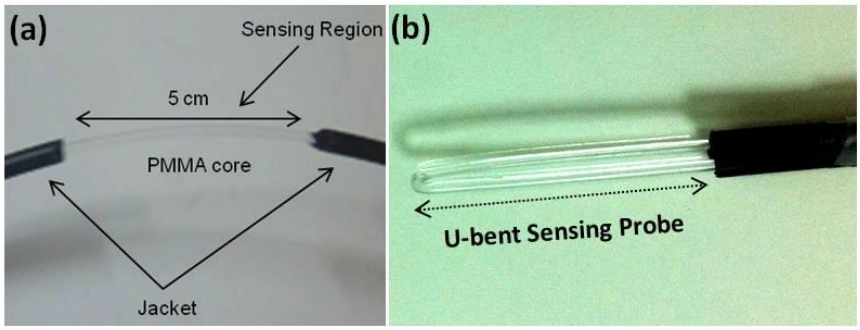

Figure 1. (a) POF after removing the jacket and cladding and (b) the U-bent sensing probe.

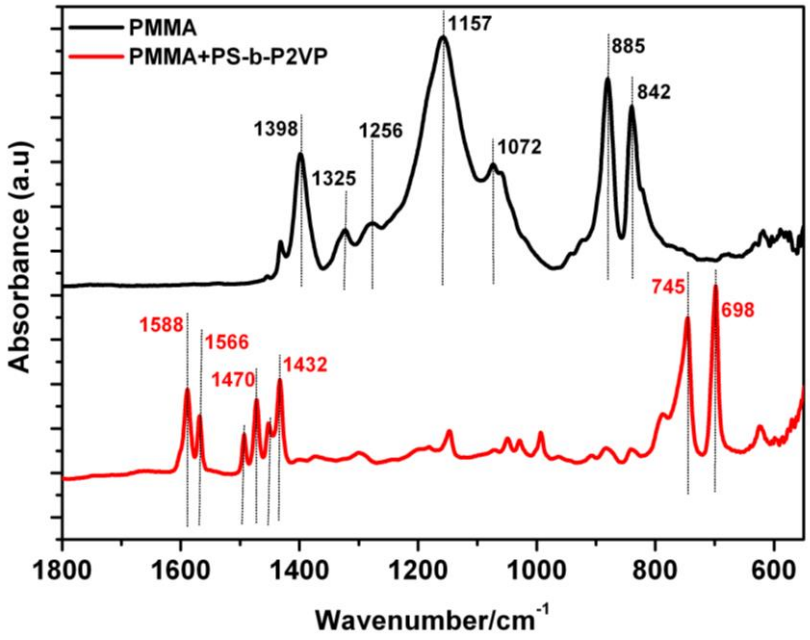

Figure 2. ATR-FTIR spectra of the PMMA core of the sensing probe before and after the deposition of the block copolymer material.

2.3 Experimental Setup

The evaluation performance of the active materials was conducted using large core PMMA polymer optical fibers demonstrating also the potential of low cost implementation of refractometric based biosensors, taking advantage of such novel sensitive materials. The optical platform consists of a U-bend multimode polymer optical fiber (POF) (ESKA GH-4001P, Mitsubishi-Rayon Co.), with an overall fiber diameter of 1 mm, and a core diameter of 980μm. The core of the POF is polymethylmethacrylate (PMMA, n_{core}=1.49), while the cladding is fluorinated polymer (n_{clad}=1.40). The light source used is a LED operating at 650 nm with maximum output power of 1 mW. The power meter used in the current work is a Newport model 2832-C Dual Channel equipped with detectors model 818-UV. The experimental set-up used for the experiments is similar to our previous relative work[9] and is shown in Fig. 3.

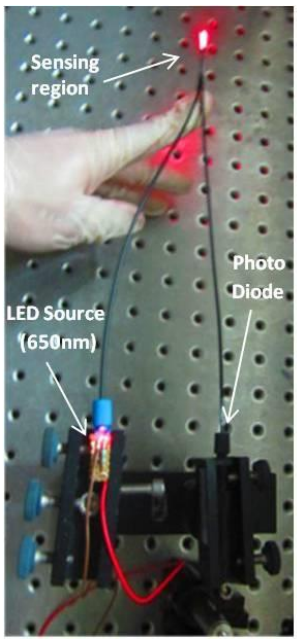

Figure 3. The experimental set up.

2.4 Sensing Mechanism

Generally, every molecular interaction is determined by a combination of the basic physical forces like hydrophobic interaction[15], electrostatic interaction[16], hydrogen interaction and van der Waals forces[17]. In this case we take advantage of the electrostatic interaction which is generated due to the opposite charge of the sensing material and the detectable protein. The sensing mechanism is based on the interaction between the evanescent field and the copolymer, which becomes stronger due to increased losses of propagation light after the bending of the active area of the POF. The detection method relies on successful adsorption of the proteins through the sensing materials which is accomplished due to strong electrostatic attractive forces generated between the protein molecules and the block or random copolymer material. This procedure increases the thickness of the deposited layer and causes variations in the refractive index at the outer material interface, leading to significant changes in the output guided wave light.

3. RESULTS AND DISCUSSION

ATR-FTIR analysis and the responsivity measurements were performed in order to evaluate the aforementioned copolymers. Both PS-b-P2VP and PS-r-P2VP materials revealed that BSA, which is negatively charged at neutral pH, was adsorbed on positively charged top-layered material surfaces with a fast initial rate and large adsorbed amount, due to the complimentary charge characteristics of the substrate and the positive charge of the sensing materials. The opposite observation was made for lysozyme which is positively charged and hardly adsorbed onto the positively fiber charged surface, due to charge repulsion. In particular, the ATR-FTIR analysis of the sensor probe (Fig. 4) after the experimental procedure showed the presence of bands associated with adsorbed BSA (amide bond frequencies at 1655, 1537 and 1403 cm^{-1}), while the absence of LYS peaks proved the low adsorption of the particular protein by the copolymer materials. This results indicate the efficient adsorption of the BSA onto the copolymer coating overlayer and prove the detection capability and selectivity of the proposed fiber sensor towards specific charged proteins.

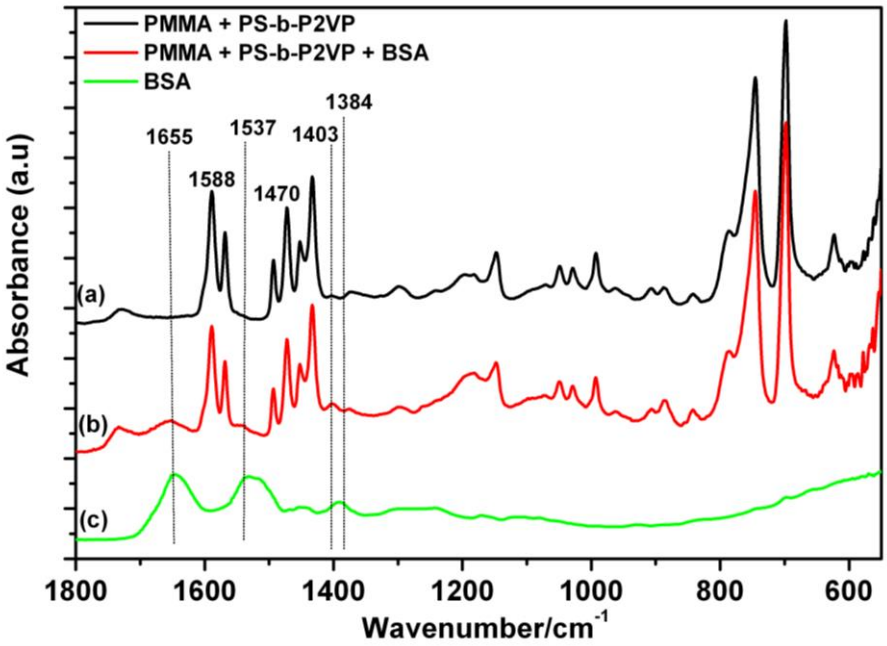

Figure 4. ATR-FTIR spectra of the sensing probe with the block copolymer coating overlayer before (black line) and after (red line) BSA adsorption, and the ATR-FTIR spectrum of BSA (green line).

Accordingly, the sensor was tested in successively diluted BSA and LYS solutions with different protein concentrations in order to determine the responsivity of the sensor and the detection limit, which in the case of biomolecular sensing, is the minimum amount of analyte that the sensor can accurately quantify[14]. The used proteins solutions were kept at constant pH in order not to affect the active net charge of the overlayer, which generates due to the variation of the pH index, simulating in parallel the human fluids at least in acidity (neutral pH). Successive response measurements over time (Fig. 5a) showed excellent repeatability in the case of the buffer and distilled water, while the detection limit was found to be 0.5 mg/ml. Fig. 5b shows the response of the sensor in BSA and LYS, using as sensing material the PS-b-P2VP block copolymer and Fig. 5c, d show the responsivity of the corresponding random copolymer Ps-r-P2VP coated on fibers, which were followed by different chemical treatments. One of the major issues is the optimization of the sensor design to improve the detection limit, working for example with fibers such as taper POF in U-bend scheme as it has been shown recently[18]. Nonetheless, this detection scheme proved to be suitable for easy, fast and low cost biosensing applications.

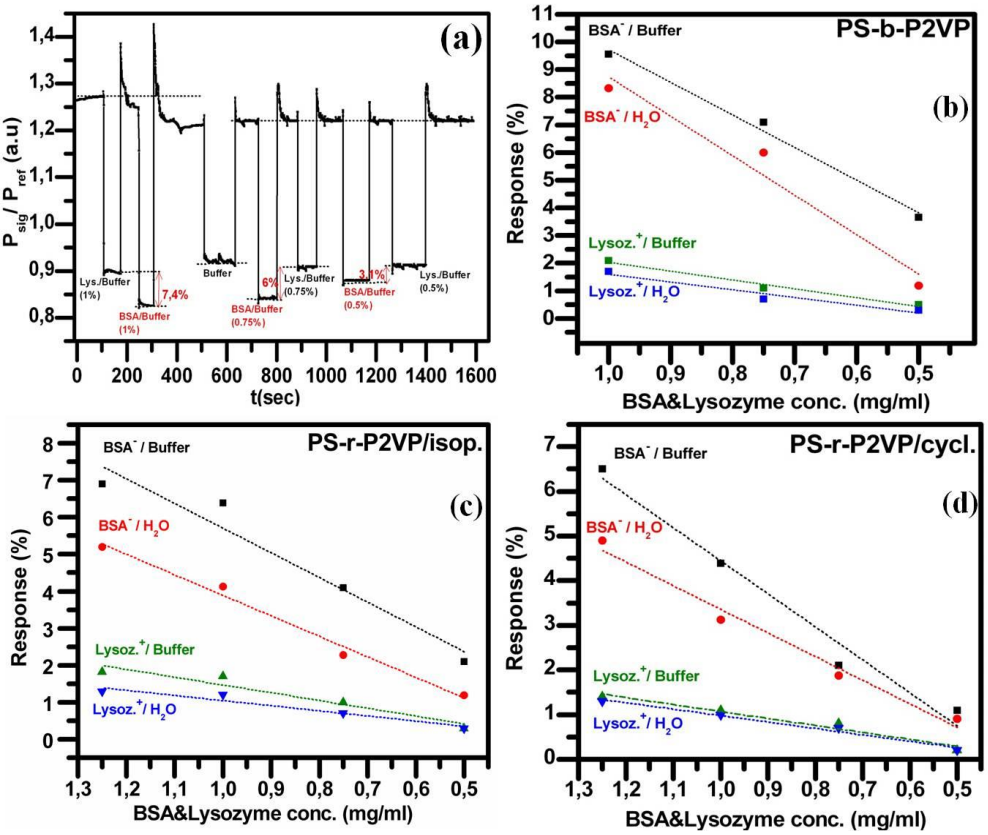

Figure 5. The experimental process indicates the relative response of the POF sensor over time in different concentrations of BSA and LYS, the absolute responses of the (b) PS-b-P2VP and (c), (d) PS-r-P2VP materials in similar protein concentrations using isopropanol and cyclohexane in the chemical treatment, respectively.

In particular, the block copolymer PS-b-P2VP showed sensing capability up to 10% in a concentration range of BSA about 0.5-1% (wt/v) with an almost instantaneous response time, due to the electrostatic nature of the interaction described, while the response of the sensor in LYS did not exceed 2% (Fig. 5b). The corresponding random copolymer PS-r-P2VP showed comparable results (up to 7%) in detection of BSA as is shown in Fig. 4c, while the response of the sensor in similar concentration of LYS was similarly relatively low, indicating low levels of lysozyme adsorption from the copolymer material in both cases. The different chemical functionalization/treatment of the fiber surface seems to affect the responsivity of the sensor mainly in low concentrations of BSA, as it is shown in Fig. 5c, d, where the hydrophilic PMMA fiber proved to be more suitable, regarding the responsivity of the sensor, probably due to the more efficient deposition of the copolymer on the fiber surface. Moreover, the increased response in the case of the buffer solution gives an added value to the tested sensor, indicating the functionality of the sensor in biological fluids.

From these observations it can be concluded that the electrostatic interactions, which govern the adsorption process, vary for the two investigated proteins.As a result a larger amount of BSA is adsorbed. Generally, the control of protein adsorption is not easily feasible because it is necessary to know the physicochemical properties of the block copolymer multilayer films which are formed onto the fiber surface. However, it is clear that these differences in the sensor response can be attributed to electrostatic phenomena. Although, as stated by the results, this method is inherently limited in both sensitivity and effective range comparing the aforementioned complex techniques, there are advantages regarding the rapidness, simplicity and the inexpensive procedure of detection.

4. CONCLUSIONS

Given the different response of a specific block and random copolymer useful conclusions can be extracted on the feasibility of their use and especially towards low cost detection schemes. The use of copolymers essentially induces a positively charged coated PMMA sensing region that could adsorb strongly negatively charged BSA. In contrast, as it was anticipated, positively charged lysozyme was adsorbed in small, but still detectable amounts, demonstrating thus an intrinsic electrostatic discrimination and selective adsorption mechanism. The optimum response's dynamic range in various BSA concentrations estimated for the PS-b-P2VP lie in the range of 0-10%, while PS-r-P2VP revealed comparable responses reaching the dynamic range of 0-7%. The chemical functionalization study of the surface sensor revealed different optical responsivity, allowing the determination of the optimum experimental procedure, using such copolymers, concerning the detection of the studied proteins. Furthermore, the reversibility of the sensors was tested when returned in buffer and H_2O solutions with zero concentrations of proteins, after being cycled through a wide range of concentrations, verifying in this way the sensors capability and stable operation.The minimum detectable protein concentration was proved to be 0.5 mg/ml. Block copolymers often require laborious synthetic techniques for their production in contrast to random copolymers that are cheaper and easier in their production. The inexpensive and design flexible POF platform, and the study of the chemical surface treatment in combination with the adaptable properties of copolymer materials led to the development of a functional, rapid and inexpensive scheme for bio-detection.

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