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Short communication

Tapered plastic optical fiber-based biosensor – Tests and application

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ABSTRACT

Cells detection is crucial in microbiological analysis of clinical, food, water or environmental samples. However, currently employed methods are time consuming. Plastic optical fiber (POF) biosensors consist in a viable alternative for rapid and inexpensive scheme for detection. In order to study the sensitivity of tapers for microbiological detection, geometric parameters are studied, such as the taper waist diameter since the formation of taper regions are the key sensing element in this particular type of sensors. In this study, a series of POF taper sensors were prepared using a specially developed tapering machine, and the dispersion of geometric dimensions is evaluated, aiming to achieve the best tapering characteristics which will provide a better sensitivity on the sensor response. The fiber tapers that presented the finest results were those constructed in U-shaped (bended) configurations, with taper waist diameters ranging from 0.40 mm up to 0.50 mm. These fiber tapers were used as the main section of the monitoring device, and when chemically treated as immunosensors for the detection of bacteria, yeast and erythrocytes.

1. Introduction

Fiber-optic sensors have been widely investigated as sensors for chemicals, and physical properties. Among them, tapered sensors in glass multimode optical fiber are well established in applications such as gas detection, spectroscopy and detection of biological systems (Ferreira et al., 2001; Leung et al., 2008; Chua et al., 2011). However, this technique has not been broadly applied to polymeric optical fiber (POF) to be used as biological sensors, but mainly as physical sensors (Merchant et al., 1999; Fixe et al., 2004).

Fiber-optic sensors can be combined with antibodies which are able to recognize and bind to a defined antigen, which induces immediate environmental changes, such as the refraction index (RI), around the probe containing the antibody. Also, the large diameter of POFs facilitates installation and alignment, unlike their glass counterparts in which a few microns misalignment results in heavy losses. Other well-known advantages are the efficient light coupling owing to the large numerical aperture, high ductility, low cost of production and easy handling.

The main objective of this study is to determine the optimum dimensions of the POF taper that best suits a biosensor probe for cells detection. We analyzed the taper performance as an RI sensor making the numerical aperture and bending radii constant whilst the waist diameter varied for both straight and U-shaped fibers. Several tapers were manufactured by means of heating and traction, using a piece of equipment developed for this goal. The tapers were then evaluated and used as an immunosensor system, which detected bacteria, yeast and lamb erythrocytes.

The operating principle of the taper is based on evanescent field (EF), given by the well known equation (Mizaikoff, 2003):

$$d_p = \frac{\lambda}{2\pi\sqrt{n_{co}^2 \sin^2 \theta - n_{cl}^2}} \quad (1)$$

where d_p is the depth of penetration of the EF, λ is the wavelength of transmitted light, θ is the incident angle of the light ray at the core/cladding interface, n_{co} and n_{cl} are, respectively, the RI of the core and the cladding. The EF is enclosed into the cladding, but by decreasing the fiber diameter by the tapering process, the EF becomes exposed outside the fiber, and consequently it interacts with the measurand.

In literature, a variety of studies approach the application of straight silica optical fibers in the manufacture of tapered sensors; nevertheless the employment of U-shaped tapers in POF can afford several advantages, such as increased sensitivity, smaller taper length, economic use of reagents, an improved handling and fabrication, and a greater mechanical resistance (Ferreira et al., 2001; Frazão et al., 2008; Nazaré et al., 2011).

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2. Materials and methods

The fibers used in this work are multi-mode, step index POF type Mitsubishi Eska GH 4001. They are POFs with a pure poly-methylmethacrylate (PMMA) core and a doped low-index-of-refraction fluoropolymer as cladding. The fibers present 980 μm of core diameter and a 20-μm-thick cladding.

Due to the softening temperature of the PMMA be around 70–80 °C, it is very easy to draw it into a taper. Although it has been reported that in a hot drawing method on finished POF the fiber breaks before any elongation occurs (Merchant et al., 1999), we have produced several tapers using a drawing machine designed for taper production.

About 200 tapers, with 14 mm length in average and waist diameters in the range from 0.28 to 0.60 mm, were obtained using a developed tapering machine. Half of the produced tapers were heated at 90 °C and bent around a mould to produce U-shaped tapers. All taper ends were cleaved and polished for a better light coupling and then have their dimensions measured.

Tapers with waist diameters in the range of 0.40–0.50 mm showed good sensitivity to RI variations whereas those with waist diameters above 0.55 mm and below 0.30 mm did not demonstrate substantial sensitivity and were discarded. Additionally, too thin fibers cannot be easily handled being extremely fragile.

The minimum RI to be measured is that of the pure water (1.33) and the maximum RI is that of pure bacteria. Therefore we must first know the RI of pure bacteria in order to properly design and adjust the electronics that will control the detection system. For this 1 l of fresh culture of *Escherichia coli* was centrifuged and dried out to extract most of the water and then measured by an Abbe refractometer producing an RI = 1.39.

In order to calibrate the sensors and measure its sensitivity we prepared several solutions of sucrose in ultrapure water with RI gradually increasing from pure water: 1.33, 1.35, 1.36, 1.37, 1.38 and 1.39.

The electronic setup shown in Fig. 1 consists in a LED (GaAlAs, 880 nm, model SFH485P) coupled to a photodetector (PIN photodiode model-SFH217F) through a 15-cm-POF with the taper. Both the LED and the photodetector are embedded into a black plastic housing, so as to improve light insulation from the environment and also acting as a mechanical fixing. The photodetector detects the light producing a photocurrent which is amplified by a transconductance amplifier built around an operational amplifier (AD8032-Analog Devices). The output voltage of the latter is fed into the microcontroller (PIC16F876A) A/D input port; its output data is finally sent via USB to a LabVIEW@ software (National Instruments) running in a microcomputer. Fig. 1 shows the optical setup for both straight and U-shaped tapers. For probing the U-shaped taper we used a 1-ml-becher whereas for the straight taper we used a customized glass vessel.

The taper region of each fiber was immersed methodically into water and then into the sucrose solutions. All measurements were normalized against the output for pure water and then annotated against the RI in order to plot the calibration graphs.

We applied in this study the immunocapture technique using the chemical treatment protocol determined by Fixe et al. (2004) for the antibody immobilization on the fiber surface modified as described by Anderson et al. (1997) that includes an intermediate layer of protein A for a correct antibody immobilization.Antibodies anti-*E. coli*, anti-*Candida guilliermondii* and anti-erythrocytes (7 mg/ml) were incubated with the taper for 1 h at 30 °C. After this time the fibers were washed with sterile saline. We tested the taper previously treated with the specific antibodies for suspensions in saline solution (300 μl) containing 10^8 cells/ml of each microorganism (bacterium *E. coli* American Type Culture Collection ATCC-25922 and yeast *C. guilliermondii* ATCC-6260) and lamb erythrocytes for 50 min at 25 °C. We fixed the polyacetal support (Fig. 1, center) to a laboratory stand with a clamp. The glass flask was fixed on the top of a laboratory jack which was used to lift the flask until the taper was immersed into the sampling solution.

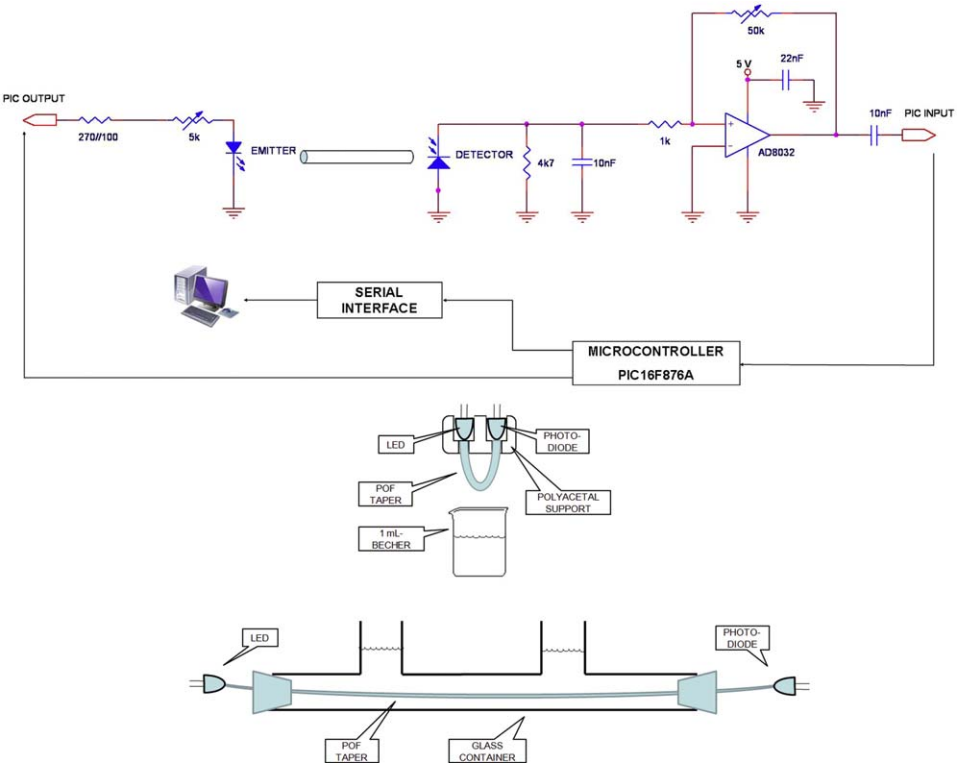

Fig. 1. Circuit diagram (top) and optical setup for U-shaped taper and straight taper (bottom).

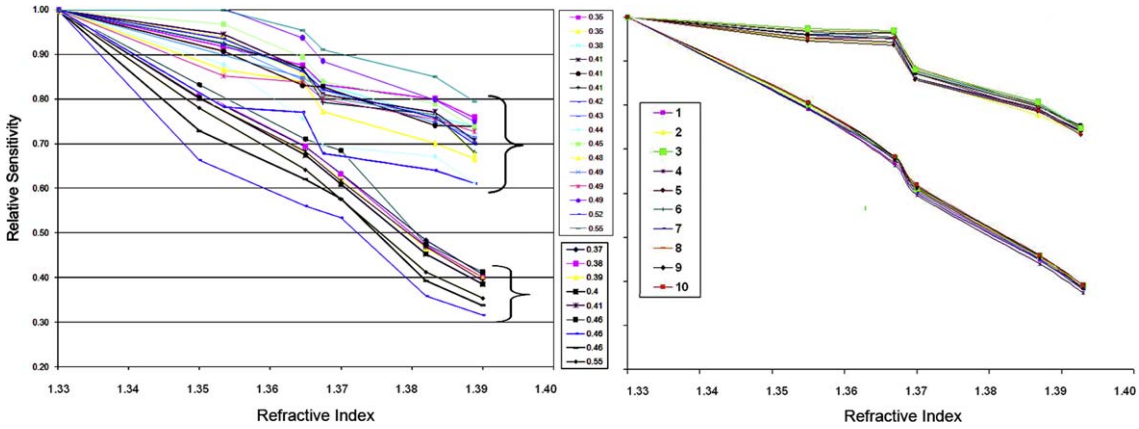

Fig. 2. Left: Response for selected straight tapers (upper group) and U-shaped tapers (lower group) under influence of different refraction indexes. Right: Repeatability analysis in a series of 10 tests between RI = 1.33 and RI = 1.39 for one straight taper (upper group) and one U-shaped taper (lower group).

For homogenization of the suspension and keeping the cells suspended avoiding sedimentation we used a 200 μl pipette manually operated to force a flow in and out of the glass flask. The pipette was operated each minute for a few seconds with a flow rate of approximately 60 μl per second.

3. Results and discussion

The taper sensor was capable to measure 1.33–1.39 or 0.06 RIU as a full scale range, being 1.33 for pure water without any bacteria and 1.39 for a suspension saturated with bacteria. For measurements in the real world the samples will be somewhere between these two figures.

Fig. 2 shows the normalized output voltage of selected tapers under the influence of different RIs, for straight tapers and U-shaped tapers.

As expected, the output light power of the fiber containing the taper is inversely proportional to the RI of the surrounding medium. The output power tends to zero when the RI of the surrounding medium tends to that of the core (RI = 1.49). Wong et al. (2003) developed and tested 120 μm tapers in 1-mm-diameter POF, however, when probing different RIs the tapers did not respond to RI in the range of 1.33 and 1.45. The reason for this may be the low sensitivity of too thin tapers due to their small surface area.The perspective of using the sensor points to a line of investigation to establish detection limits as low as possible and additional studies are being conducted in this regard. However, in the present study the main objective was to investigate the importance of geometry of the taper when analyzing a small range of cells concentration and simulate the RI of microbial suspensions with solutions of sugar. The larger the step response between pure water and water with cells with RI = 1.39 the better the taper sensitivity to small amount of cells. In comparing results shown in Fig. 2, the first thing to be noticed is the larger sensitivity of U-shaped fibers as compared to straight tapers. The reason for this is that inside a curve, the higher order modes of the guided light reach the core–cladding interface with a smaller angle (θ) consequently increasing the penetration depth of the EF. This means a greater interaction between guided light and surrounding media.

In order to verify the repeatability and stability of measurements and to exclude possible coupling and insertion errors, two tapers, one straight and one U-shaped were chosen to be tested. These tapers were repeatedly immersed into sucrose solutions with different RIs by ten times. The results, shown in Fig. 2, appear almost superimposed, therefore a good repeatability was achieved.

From these data one can calculate the sensitivities of both straight and U-shaped tapers. The average sensitivity in the range 1.330–1.393 for straight tapers is 6.1 mV/10^{-3} RIU and that for U-shaped tapers is 12.1 mV/10^{-3} RIU.

From the dispersions obtained from these experiments it is possible to calculate the uncertainty in RI measurement of both types of tapers. The average standard deviation found for straight tapers is 74 mV and that for U-shaped tapers is 17 mV. These data yield average uncertainties of 12.2×10^{-3} RIU and 1.42×10^{-3} RIU. These results are in conformity with the findings of Khijwania and Gupta (2000) when experimenting with tapers in silica fibers; they also noticed the better sensitivity of U-shaped tapers. Therefore U-shaped POF tapers show improved performance, both in sensitivity and in uncertainty.

Although thinner waist diameter theoretically allows deeper EF, thin tapers also present smaller surface area with a smaller probing capacity. These two effects probably counterbalance each other at a specific diameter. Then, for even smaller diameters, the second effect prevails decreasing the overall sensitivity.

It is important to notice that the tapers do not always present the ideal biconical shape for which one single parameter, the waist diameter, would characterize them. In other words it is impossible to account for all possible parameters such as entrance angle, length and surface area. Therefore two tapers with same waist diameter can present different behaviors. Although we excluded from our statistical analysis all geometric shapes much different from the biconical, we can notice some tapers outside the ideal curve.

When the fiber is drawn to, say, 500 μm in diameter, theoretically, the cladding would also decrease from 10 μm to 5 μm. The cladding however, being made of a different material, could also rupture in different points, which would be invisible to the naked eye. In either case, the fiber sensing characteristic provided by the tapering procedure can be related to the complete loss of the cladding (Merchant et al., 1999) or its partial removal (Guo et al., 2009). In these situations, in which the cladding thickness decreases or disappears, a greater escape of the light from the fiber is observed, resulting in a better interaction between the EF and the medium.

The lack of sensitivity of tapers with greater diameters is related to the maintenance of an intact cladding, a fact that does not permit an ideal interaction between the EF and the medium being investigated. On the other hand, in tapers with diameters below 0.30 mm it can be observed that there is an increased loss of light, since fibers with smaller diameters guide less modes. In this case a dimmer light reaches the fiber output end, needing a larger amplifier gain which causes a greater electric noise at the amplifier output.

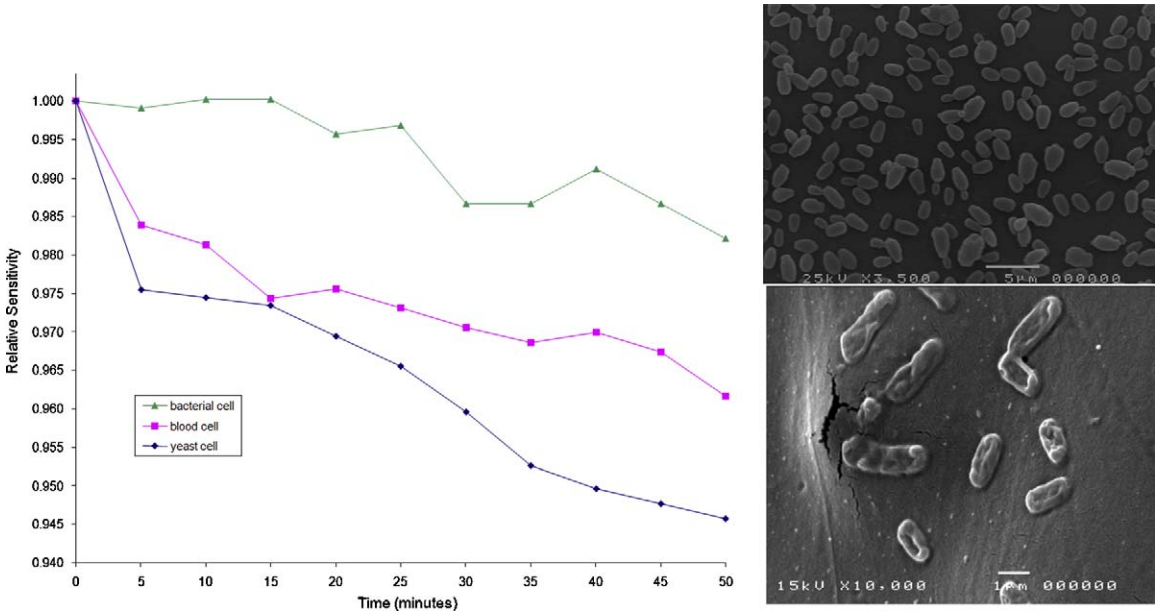

Fig. 3. Left: Sensor response for three different types of living cells: yeast cells, lamb blood cells and bacteria. Right: Scanning electron microscopy of *Candida guilliermondii* (upper picture) and *Escherichia coli* (lower picture) linked to antibodies over the taper surface.

The main goal of the next experiment was to check whether the chemical treatment protocol used by Fixe et al. (2004) in PMMA will also work for the fluoropolymer of our POF cladding. Three U-shaped POF sensor heads lying within the optimal sensitivity range, between 0.40 and 0.50 mm, were selected to be used in the experiments with living cells. The cells, when linked to their specific antibodies, promote a variation in the RI close to the surface of the taper which is then read by the system. Three experiments were performed in order to test the sensor's ability in cells detection using U-shaped fibers. Three types of cells were used: *E. coli*, *C. guilliermondii* and lamb erythrocytes. The cells attachment over the taper surface was confirmed using scanning electronic microscopy (SEM) images taken from the tapers as shown in Fig. 3.

A 10^8 cells/ml concentration of yeast cells, lamb erythrocytes and bacteria cells caused an approximately variation of 50 mV, 40 mV and 20 mV in the sensor reading, respectively, after 50 min. Fig. 3 shows the sensor response for each experiment along the time. The system showed a smaller sensitivity to *E. coli* when compared to the response caused by erythrocytes and yeasts. This is probably due to the *E. coli* smaller physical dimensions (3 μm), followed in size by erythrocytes (7 μm) and *Candida* (8 μm).

Rijal et al. (2005) developed a biochemical treatment in silica fibers to covalently bond to the surface of the tapered region a monoclonal antibody to the pathogen *E. coli*. The protocol of Fixe et al. (2004) was applied to pure PMMA, which would not necessarily work for a different polymers. However, the POF manufactures do not disclose the exact composition of the fluoropolymer material used in the cladding. On the top of that, there are no pure PMMA POFs commercially available. We used this same protocol with positive results, as shown by microscopy, suggesting some similarity between the two materials.

Although there is a clear variation when the system is tested against different types of living cells, the obtained sensitivity cannot be considered suitable for commercial biological measurements yet. However, this study did not aim to develop a final protocol to improve the detection limit of the system to levels applicable in the field, but to determine the best setup and fiber taper to be used in the biosensor. Additional studies are being conducted in order to increase the sensitivity of the system, particularly at the optoelectronics section.

4. Conclusion

The POF technology employed in the construction of the biosensor fulfills the requirements of ease handling and simple construction. It was observed that the geometry of the taper interferes in the behavior of the sensor. Both straight and U-shaped tapers were sensitive to the surrounding environment when produced in diameters between 0.40 and 0.50 mm. However, U-shaped tapers have shown better sensitivity and are more practical as they fit easier in smaller sample flasks. The manufacturing of the fiber tapers was accomplished by a specially designed drawing machine which is of simple use and of inexpensive construction.

The sensitivities of tapers in the RI range of 1.330 and 1.393 for straight tapers and U-shaped tapers are approximately 6.1 mV/10^{-3} RIU and 12.1 mV/10^{-3} RIU, respectively. The uncertainties in RI measurements were 12.2×10^{-3} RIU and 1.42×10^{-3} RIU respectively, indicating the U-shaped tapers as those with better sensitivity and uncertainty.

The use of U-shaped POF tapers chemically treated with immobilized antibodies, together with a simple optoelectronic system, enables the detection of target cells indicating the POF biosensor as a potential device to detect cells in aqueous medium.

Acknowledgments

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