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High Sensitive Biosensor for *in vitro* Detection of Patulin Based on Optical Planar Waveguide

Mycotoxins present a concern to human health due to its adverse human health effects which ranged from poisoning to cancer and immune deficiency. Therefore, accurate and sensitive quantitative detection methods are of primary importance to limit their risk. This work aims to provide a high sensitive biosensor for the in vitro detection of mycotoxin (in particular Patulin) using optical planar waveguide and highly specific bio receptor. In this system, a polarization controlled laser traveling through the waveguide core and the sensor working as a polarization state detection, via observe the phase shift between s- and p-components of propagation light which caused by the molecular adsorption at the core-cladding interface. The refractive index sensitivity of this experimental setup has reached~9600RIU/rad, which is considered one of the highest values have demonstrated. This sensitivity value enabled this system to detect mycotoxin molecules in extreme low concentrations in range of 0.01 ng/mL.

Keywords: Biosensors; Optical waveguide; Mycotoxins; Refractive index
Received: 01 June 2024; Revised: 10 August 2024; Accepted: 17 August 2024

1. Introduction

Nowadays, the detection of toxic pollutants is the main task of environmental science efforts, agriculture, security and food industry. There is increasing interest in detection of mycotoxins because of their toxic and carcinogenic effects on both human and animal [1]. International organizations concerned with public health and safety environment have set strict limits on mycotoxin levels in feed and food [2]. Mycotoxin molecules size in range of a few hundreds of Daltons, as a result detecting them is not easy especially at low concentration levels. High-tech detection instruments such as mass spectroscopy and HPLC have ability to achieve the required sensitivity. However, these equipment are not suitable for work in field and combating the spread of these toxins due to its high cost the need for skilled technicians to work on it. Therefore, there is a global demand to develop easy to use and portable biosensors for mycotoxin detection [3]. Optical waveguide in mode of polarization interferometer is considered one of the proposed technologies to provide the appropriate biosensor. The waveguide is characterized by the ability to repeat sensing mechanism through repeat the light reflection at the bio-receptor element, which give possibility to amplify the response [4- 6]. Recently, there are several successful optical biosensors based on optical planar waveguides have been demonstrated [7- 9]. The most development of optical planer waveguide based biosensor devices lies main two techniques; ring resonators [10-12] and Mach–Zahender (MZ) interferometers [13-15], both methods have achieved high refractive index sensitivity in range of 8000 rad/RIU. Fully silicon waveguide biosensor developing is an attractive aim, and It is a step towards fabricate a highly sensitive portable biosensors for in-field analyses of interest, and meet the need for a safe environment from mycotoxin contamination. The main idea of this work is an attempt to present a low cost, high efficiency design that is easy to implement and basis for provide a highly sensitive integrated biosensor possesses ability to detect low molecular-weight molecules particularly a mycotoxins molecular, even in low concentrations (ppt range). In general, typical biosensor consists of two main parts: 1) Biorecognition element (bioreceptor), to specifically recognize and select the analyte, which can employ various biological materials such as antibodies, enzymes, nucleic acids for this purpose . 2) Transducer element, which converts any physical or chemical change a result of capture of target molecules to a measurable signal [16-19]. In this work, a planer optical waveguide immunosensor using a novel principle of polarization interferometry has been adapted to detect kind of mycotoxin molecules named a patulin. This type of mycotoxin poses a public health concern as it is widely spread in environment, particularly food storage [20,21]. The “Codex Alimentarius Commission” and WHO were have listed standard limit exposure to most kinds of mycotoxins [22-23]. Therefore, developing a patulin sensor supports combat their spread and reduce their risk.

2. Planar Waveguide Design and Biosensing

The optical planar waveguide being the key element of this biosensor was built on silicon layer using microelectronic techniques consisted of thin layer of Si_3N_4 (200 nm) serve as a waveguide core, surrounded by silicon oxide layers (3 µm) (Fig. 1). The number of reflections per unit length during propagate the light through the waveguide core directly affects the refractive index sensitivity of the system. The large difference between the refractive indices of silicon nitride (core) and silicon oxide (clads) allows light to propagate through the core at a reflection angle of 47°, Which allows to the largest possible number of reflections per unit length, during

propagation the light through the core of the waveguide, According to the calculations that took into account Good–Hänchen effect, for this waveguide structure the number of reflections is approximately 3000 reflections per millimeter [25, 26].

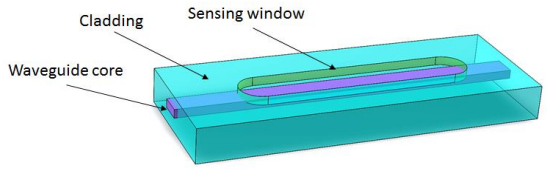
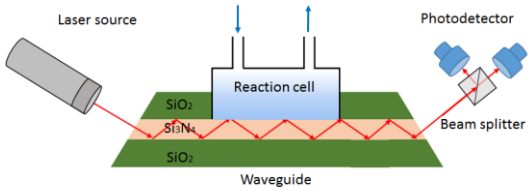


Fig. (1) The optical waveguide structure and sensing window

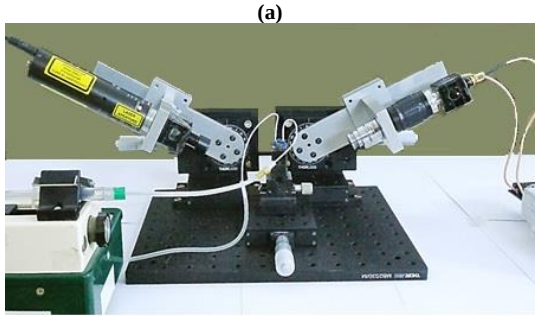
When polarized light reflects from an interface between two media as total internal reflection, the s compound reflect directly from interface boundary, while the p compound penetrates a little into the second medium before it is reflected. Delay p-compound for s-compound affects their synchronization status which determine the polarization state. The amount of delay H from S and therefore the change in the polarization state depends on the refractive index of the second medium. Therefore, the state of polarization could be a function of the change in the refractive index of the second medium. The principle of the optical sensing of this system depends on the change in the refractive index due to the bioreaction. When the bio-molecules interact in a medium serves as a cladding to the waveguide core, the refractive index changes as a result, this change leads to a shift between p and s compound of the reflected light. This p-s shifting changes the polarization state of the light, which the system records it as a response signal. The p-s shift is proportional to the change in the refractive index and therefore to the concentration of the reactants. The bio reaction that occurs in this process is that occurs in this process is the binding of target biomolecules to the receptor elements. Therefore, by monitoring the polarization state of the output light from the waveguide, the concentration of the targeted bio molecules in the sample that is injected into the cell reaction can be determine.

LDM635 compact Laser Diode 5mW, 635nm, Thorlabs company product has been employed to provide the waveguide with the required laser waves, a polarized controlled 635nm laser was applied at polished slant edge via a microscope lens and set of focusable cylindrical lenses, to achieve maximum light-core coupling efficiency and minimize back reflection, The proper light polarization condition has been achieved by using 1/4 plate (CP1L633-Left-Handed Circular Polarizer, THORLABS product), followed by a linear polarizer (LPUV050-MP2, Linear Polarizer, THORLABS product). In the upper side of the waveguide 6 mm length from the cladding layer has been removed (using wet etching of silicon oxide) to open "sensing window" that allow the bioreaction being in contact with the optical system (specifically with waveguide core).

In the experiment setup in Fig. (2), laser beam applied to the waveguide core via slanted edge, then collected with two photodiodes at the other side, during its passage through the core, it comes into contact with the reaction cell via the sensing window. There is also an electric syringe connected to the reaction cell to inject the sample solutions and deliver biomaterials into the reaction cell (Fig. 2). Dark nylon reaction cell was sealed onto sensing window, so injecting the biomolecules into the cell allowed to deposit them on a Si_3N_4 surface (Fig. 3).



(a)



(b)

Fig. (2) Experiment diagram (a), experimental set-up (b)

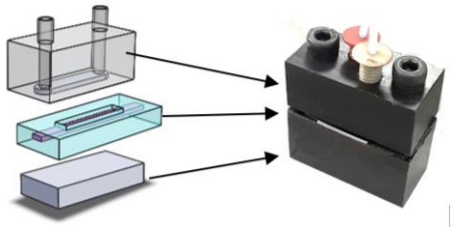


Fig. (3) The waveguide and reaction cell, (SOLIDWORKS 3D Software)

The output light beam is split into two beams, one of them is directed through a linear polarizer (PVAE1-A, Thorlabs Company) to a silicon photodiode (No.1) (SM05PD2A, Thorlabs Company) while the other is directed directly to a second photodiode (No.2) (Fig. 4). The polarizer converts the changes in polarization (which accompanies the refractive index variation) into changes in intensity so that, therefore, the photodetector (No.1) signal is related to the polarization state, while the photodetector (No.2) signal refers the laser intensity variation (due to reasons unrelated to polarization changes), this signal use to eliminate the irrelevant variations of the light intensity, which is not relevant to the polarization

rotation. So, processing these two output signal give clear indicator to the changes polarization state. The typical response to the change in the refractive index of the medium (after filtering out the noise and eliminate the effect of light intensity variations that was not relevant to a change in phase) show clear signal can be processed via software to extracting the amount of phase shift (Fig. 5). The sensitivity of the system is determined by the amount of phase shift to refractive index unit.

Fig. (4) Output signal acquisition

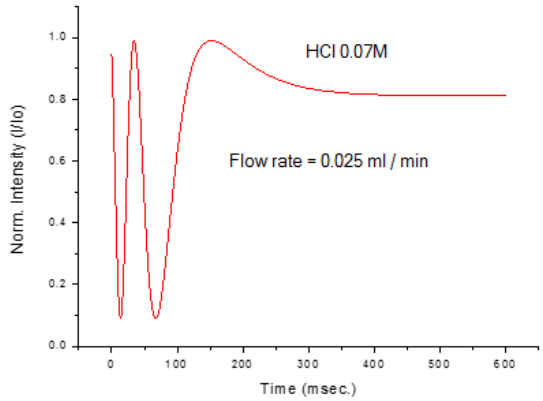


Fig. (5) Real-time monitoring of response signal to the change in the refractive index, (Origin 8 Data Analysis and Graphing Software)

3. Testing Waveguide Sensor

Preparing this system to work as a bio or chemical sensor requires determine the sensitivity of the system to the change in refractive index. RI sensitivity was evaluated experimentally by injecting a standard refractive index aqueous solutions and record the response, for this purpose, different concentrations of hydrochloric acid 0.03M, 0.05M, 0.1M, 0.2M, 0.3M, 0.4M, and 0.5M have been used as test mediums (table 1) [25,26]. Varying the refractive index of the liquid medium was resulted a multiperiodic output signal waveforms. The phase shift associated to each change in refractive index was calculated by using proper software shown in table (1). The period's number was linearly proportional to the increment in the refractive index as shown in Fig. (6), these results allow to calculate the refractive index sensitivity of the system according to [7]:

$$RIS = (2\times\pi\times N)/\Delta n \quad (1)$$

where N, Δn represents the period's number and the change in refractive index respectively

The sensitivity curve shows the experimental refractive index sensitivity is ~9600 rad/RIU, (Fig. 6). The noise in the output signal was less than 5%, so that this system can track the change in the refractive index of the sample in the range of 10^{-4}. These results of optical performance are enough to employ the system for biological sensing.

Table (1) Response signals to HCl solutions

Solutions	n	Number of periods
HCl 0.03M	1.3332 ± 0.0001	01.0
HCl 0.05M	1.3334 ± 0.0001	01.5
HCl 0.1M	1.3338 ± 0.0001	03.0
HCl 0.2M	1.3348 ± 0.0001	06.0
HCl 0.3M	1.3357 ± 0.0001	08.6
HCl 0.4M	1.3366 ± 0.0001	11.5
HCl 0.5M	1.3376 ± 0.0001	14.6

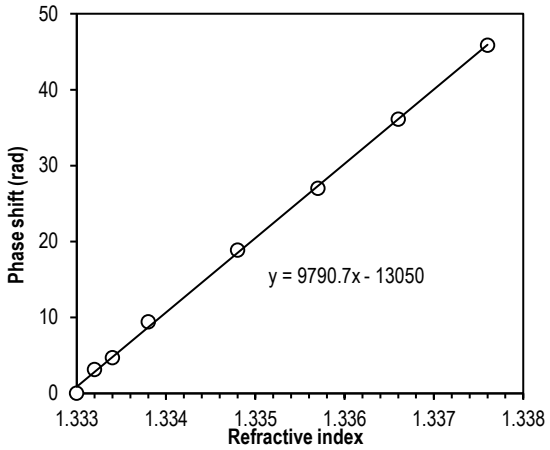


Fig. (6) Phase shift response with refractive index change

4. Immuno-sensing tests for Patulin detection

Patulin is a furopyranone, 4hydroxy,4H-furo pyran-2(6H)-one, molecular formula $C_7H_6O_4$ (Fig. 7), and molecular weight 154.12 g/mol [27]. Direct immunosensor format was employed to detection of Patulin using specific antibodies for this kind of mycotoxin. The Patulin specific antibody was immobilized electrostatically on the sensing surface via two basic steps; (i) Deposit very thin layer (≈2nm) of poly-allylamine hydrochloride polymer (carrying positive charge), (ii) Deposit Protein A (at pH=7, the charge being a negatively), the polymer layer holds the protein particles due to the electrical affinity between them. (iii) Then the last step, deposition the Patulin antibodies via the binding mechanism between them and Protein A molecules (Fig. 8). The detection test of Patulin molecules was undertaken by consecutively injection of standards concentrations of Patulin (0.01, 0.1, 1, 10, 100, and 1000 ng/ml.).

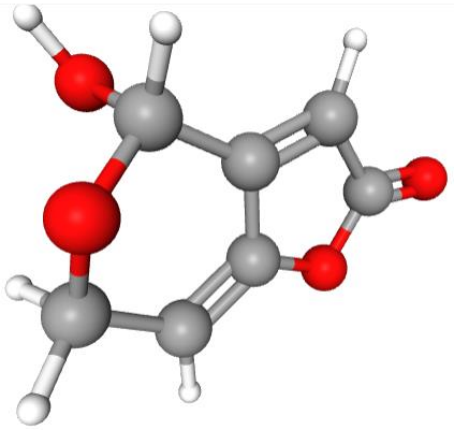


Fig. (7) 3D Patulin molecular structure [30]

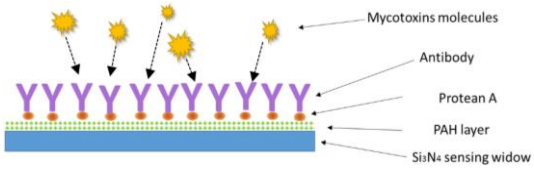
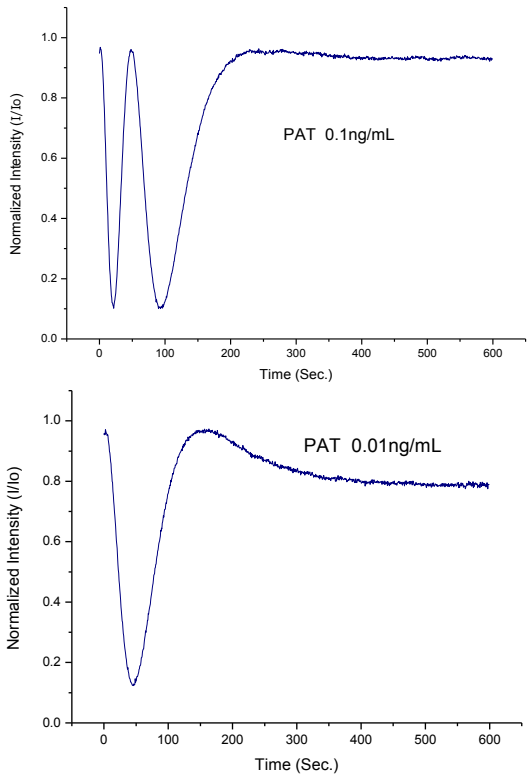


Fig. (8) Functionalization of the sensing window by immobilization of antibody to consist of a bio-receptor platform

After each concentration injection, a buffer injection is followed to wish out the unbinding molecules. The typical response signals to injections of these various concentrations of Patulin are shown in Fig. (9).



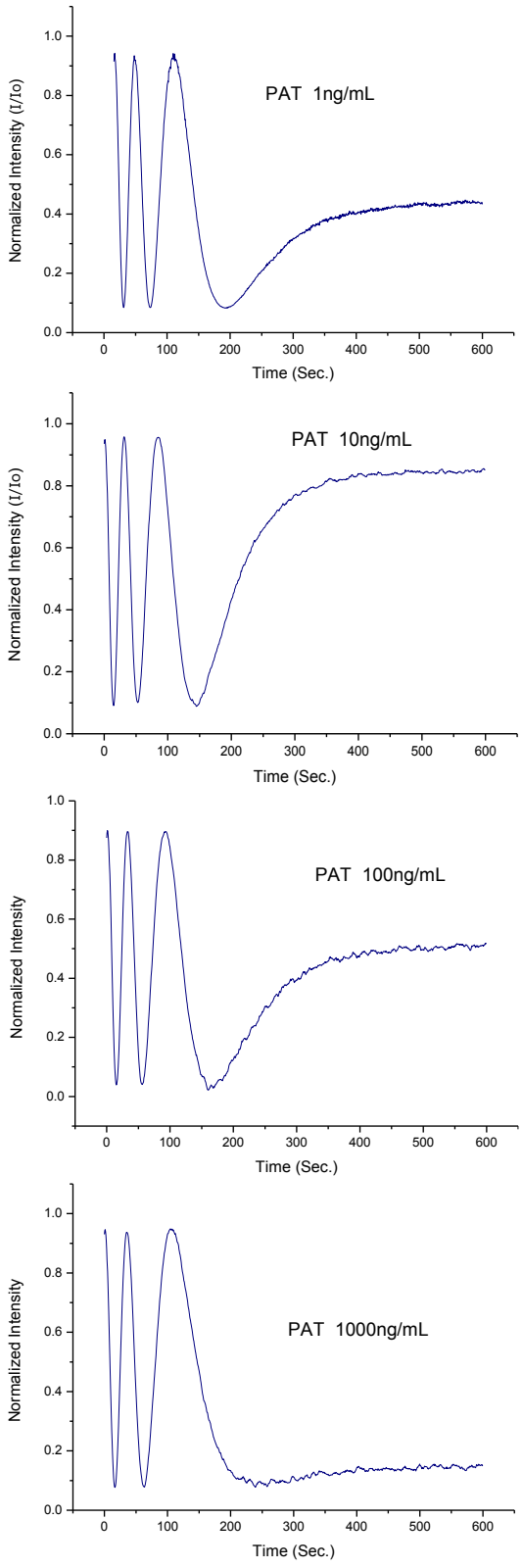


Fig. (9) Typical response signals bind different PAT concentrations to their specific antibody

Extrapolation the response curve (Fig. 10) show that, the in the beginning the response was semi linear, the number of periods of response signal oscillation is directly proportional to the increase in Patulin concentration. Then the response declines, which can be interpreted as decrease in the number of

available receptors. Continuance the sequential injections of Patulin solutions cause a saturation of the antibodies gradually (Fig. 10). Therefore, decrease of the phase shifts while continuing to inject the solution are due to beginning of saturation of the antibodies binding sites. Eventually the system reaches a complete saturation state and there will be no phase shift more. This behavior in agreement with the fact that the immobilized antibodies on the sensing surface are exhausted. The smallest concentration that has been detected in these system was 0.01 ng/mL, corresponding to 1.5 periods of signal, while in the ELISA test, the minimum is an approximately 0.8 ng/ml [28]. For each PAH concentration the total phase shifts can be estimated by summation of the responses of each single injection then subtract the background. This total phase shift vs to accumulated concentration of PAH reveals that the sensing response is approximately linear. The standard deviation (SD) of the measurements was about ± 0.5 rad which allows to evaluate the low detection limit (LDL) as 0.7 pg/ml via intersection of the response fit curve with the three times noise level which was 1.8 rad. (the inset in Fig. 10).

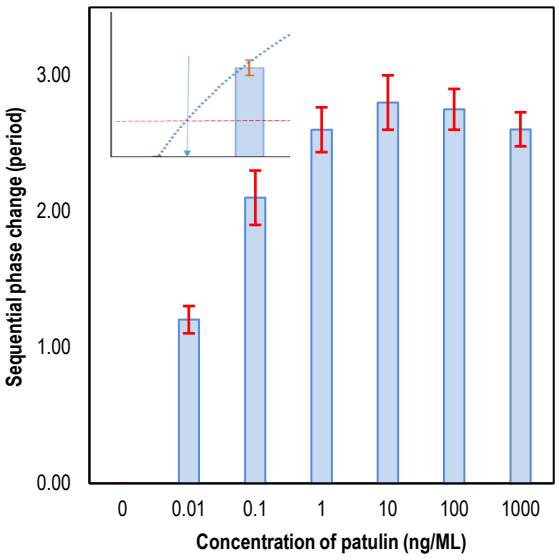


Fig. (10) Concentration a dependence of response signal caused by consecutive injections of PAT (with standard deviation of the measurements), Inset shows zoomed in section of the diagram (the red dash is a line three times noise level, blue points is the fitting curve, blue arrow points LOD value

4. Conclusion

The main aim of this work which is develop high sensitive biosensing system has been achieved. The high refractive index sensitivity of the optical system of around 9600 rad/RIU was reached that was close to value reported for one of the most advanced systems Mach Zehnder biosensors. Immune-sensing of PAT has been done by recording the responses signals induced by the consecutive injections of PAT solutions (range of 10^{-2}ng/mL to 10^{3}ng/mL). The lower detection limit (LDL) was evaluated a 7×10^{-4}ng/m, which is much less than which have been announced by other techniques such as; TIRE (0.01 ng/mL), LSPR, (0.2 ng/mL) and SPR (1.5 ng/mL), [29].

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