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Waveguide-Based Biosensors for Low-Level Concentration Fumonisin B1 Detection

Optical phenomena such as polarization, phosphorescence, fluorescence and interference have achieved great success in the field of bio sensing applications. Optical waveguides biosensor system are constituted of material possess high refractive index and high permittivity surrounded by lower refractive indices materials as cladding or media to be sensed. This arrangement give rise to light propagates across the core of the waveguide via total internal reflection, during this propagation, evanescent field arises around core in the cladding area and decreases exponentially as away from the cladding-core interface. This phenomenon has been exploited in this work to detect the biological molecules. Presence of the targeted biological molecules in the evanescent field area effect on the parameters of the reflected light beam particularly the polarization state. This mechanism of sensing has been applied in this work to detect to detect mycotoxins molecules. The results showed the success of this approach and its ability to sense very low concentrations in range of 0.01ng/mL, which meets and exceeds the required detection limits for environmental safety.

***Keywords*:** Optical waveguides; Biosensors; Immunosensing; Mycotoxins
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1. Introduction

Total internal reflection is a simple optical phenomena was provided many beneficial application in the field of bio sensing. Optical waveguides are one efficient means to exploit this phenomenon for biosensing [1-3]. When the light applied at the waveguide core, it will be confined and guided within the core region via repeated total internal reflection process (Fig. 1). However, the electromagnetic waves of the propagation light penetrate and transcend little bit (in range of half wavelength) the boundary between the core and cladding layer [4-7].

The intensity of the field across the boundary decrease exponentially with the distance from core-cladding interface, and completely falls off at a less one-half the wavelength distance. This slight penetration into the media close to the core in which the sample placed makes the propagation light come into contact and react with the sample during each total internal reflection event. The presence of target molecules can affect the optical properties of light, when the light properties observed, can be inferred from it the percentage of target molecules presence. This sensing can be done via several transduction approaches include refractive index changes, fluorescence detection, spectroscopic shifts detection [8-12]. The current work focused on monitoring the change in polarization state of the propagation light. Since two decades researchers interested in methods of bio sensing based on optical waveguide.

The idea of evanescent field was initially demonstrated by Tiefenthaler and Lukosz when they discovered the variations in the propagation mode angle due to change in the humidity. After that, these researchers proposed application of this observed effect as to biochemical and chemical sensing [13-16]. Optical biosensor systems based on both multimode and single mode waveguide are considered one of the most useful research areas in the field of bio sensing. Biosensor used for measurement the biomolecules concentration should be achieve certain required performance which are quite different from other typical sensor. Particularly, a biosensor should be characterized by several features, such as sensitivity, selectivity, and ammunition to the external disturbances such as temperature and pressure changes. Photonic technologies make these requirements possible.

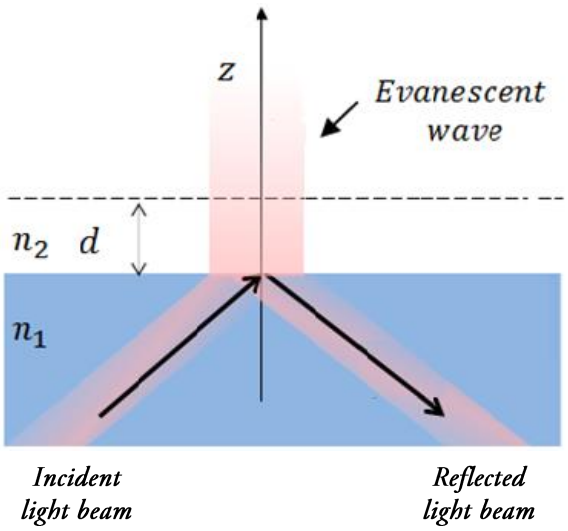


Fig. (1) Evanescent filed propagating along the two mediums interface

Mycotoxins molecules typically are detected by immune sensing approach, which is done by binding

the targeted molecule to particular bio receptors like aptamers or antibodies [8-10]. Optical immune sensing approach including spectroscopic ellipsometry, surface plasmon resonance and optical wave guides are the most common and the most proper technique. The method of measurement the phase difference between polarized light components s and p via ellipsometer has provided a high detection sensitivity for the mycotoxins that corresponding well with what is required [17]. However, this TIRE method based on expensive and bulky optical instrument (spectroscopic ellipsometer). In this work, it was presented easier method to track the p-s shifting, by use the optical waveguide structure as a mean of polarization interferometer. The targeted molecules for detection in this work are one type of mycotoxin called Fumonisin B1. It is considered amongst the most toxic types of mycotoxins which produced by kind of fungal called Fusarium verticillioide and Fusarium proliferatum [18-21]. FB1 cause widespread contamination particularly in wheat, rice, and their products, so it poses a terrible health risk. The Fumonisin B1 molecule is small, so its detection, especially at low concentration levels, is a challenge. Therefore, the success of the current experiment in detect it is considered a valuable achievement.

2. Experimental Setup

Optical planar waveguide (Fig. 2) is a key part of the biosensor which proposed in this work, this optical system has been built on silicon wafer using standard microelectronic processes. This system consists of a core in the form of 200 nm thick, 1 mm width and 6 mm length channel of silicon nitride (Si_3N_4) surrounded by silicon dioxide (SiO_2) as a cladding layer.

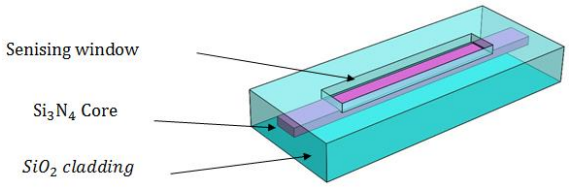


Fig. (2) Waveguide structure

A large contrast in the refractive index of the waveguide core (n = 2.02) and surrounded cladding (n = 1.46) makes the propagation angle is a steep angle (47°), which allowing to occur a large number of reflections per unit length of the light path through the channel was estimated as 500 reflection per mm. Part of the top layer of SiO_2 cladding has been removed by wet etching using hydrofluoric acid diluted (1:10), to make sensing window. Opening this window enable the sample to be in direct contact with the channel. There is also another element called reaction cell which works to contain the sample and make it in contact with the channel of the waveguide through the sensing window. Reaction cell is a rectangular cavity ($1\times1\times6mm^3$) engraved in a black nylon block equipped with inlet and outlet micro pipes fitting.

This nylon piece which contain the reaction cell fixed tightly on the waveguide so that the reaction cell (cavity) is facing tightly the waveguide sensing window (Fig. 3) to allow to the injected liquids to be in contact with the waveguide core (channel).

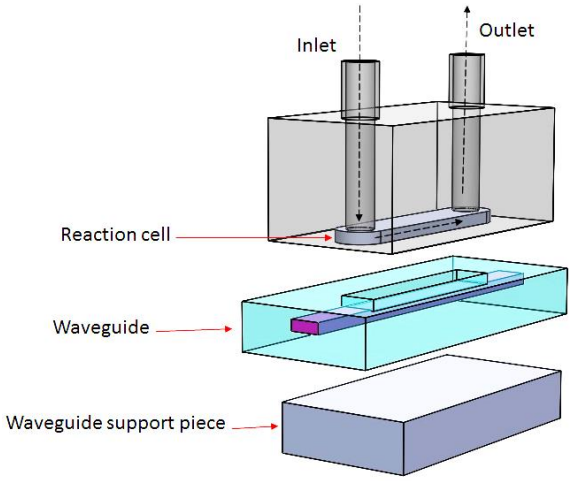


Fig. (3) Reaction cell and waveguide

On the other hand, the system requires polarization controllable laser source, this has been done using a semiconductor laser source equipped by quarter wave plate and linear polarizer. The light emitted by the semiconductor diode, which has linear polarization converted to a circular polarizer via quarter-wave plates, then, using a linear polarizer, the required polarization angle is adjusted. On the other hand, sensing the laser output and tracking the change that occurs in its physical properties requires a detection system. The detection system has contained Linear Si CCD Array (Smart Line Camera) LC100 THORLABS product (Fig. 4), and set of polarizers to modulate intensity by polarization state, and make it detectable via optical detector elements.

All these units are arranged to form a system (Fig. 5) that senses changes in refractive index, in the sample as a result of the binding the targeted biological molecules with the bio-receptor element. Therefore, the key idea of the system work is to sense phase shift between polarized light components (p and s) as a result of changes in optical properties of the sample according to the equation that governs that [22-23]:

$$\Delta\varphi_{P,S} = 2\,arctan\left[\frac{\sqrt{(N^2\,sin^2\theta-1)}}{N\sin\theta\ \tan\theta}\right] \qquad (1)$$

N is a refractive indices ratio of the core to cladding ($N=n_1/n_2$), θ is the propagation angle. Therefore, the performance of this system depends on the amount of observed p-s shift versus the change in the refractive index of the sample medium which called refractive

index sensitivity. The RI sensitivity was measured by inject different concentrations of NaCl solutions and recording the multi-periodic output signals. The results were presented in (Fig. 6), which shows the sensitivity reached 9300 rad/RIU.

Fig. (4) Linear Si CCD Array LC100 THORLABS product

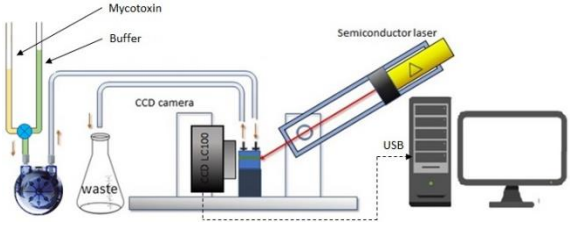


Fig. (5) Experimental setup of polarization interferometer bio-sensing

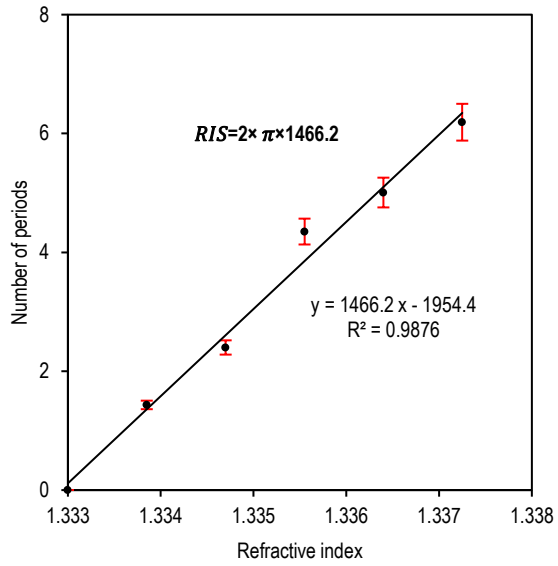


Fig. (6) Refractive sensitivity test

3. Detection of Fumonisin B1

Detection of Fumonisin B1 has been carried out by using the experimental set-up expressed in Fig. (3), which has refractive index sensitivity of approximately 9300 rad/RI unit. The detection was accomplished using direct immunosensor approach with immobilization of the specific antibodies on the sensing area via protein A immersed in layers of poly-allylamine hydrochloride (PAH) (Fig. 7).

The Fumonisin detection test has been undertaken by injecting a multiplying order concentrations (0.01, 0.1, 1, 10, 100, and 1000 ng/mL). The typical responses of the system to inject these Fumonisin concentrations of are shown in Fig. (8). It clear that the period number of the fluctuated signal increases with multiply the Fumonisin concentration. The precision of phase shift estimate was about 0.6 rad. However, continued increased the concentration cannot continue lead to continuous increase in the number of resulting signal periods. The Fumonisin B1 solution sequential injections lead to gradual saturation of specific antibodies binding sites (Fig. 9). This saturation results in a decrease in the number of periods or even their complete cessation. The beginning saturation results in a slight decrease in the number of periods, and the completely saturation resulted in a very limited phase shift because of the non-specific binding effect. This behaviour corresponds to the fact of consumption of the antibodies that are immobilised on the sensing surface. Inject buffer solution caused about 1/4 of a period signal which due to washing out the non-specifically bound Fumonisin B1 molecules, this level has been taken as a background level (baseline) for this experiment.

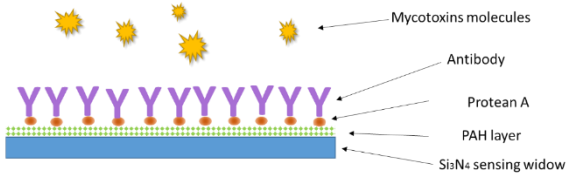
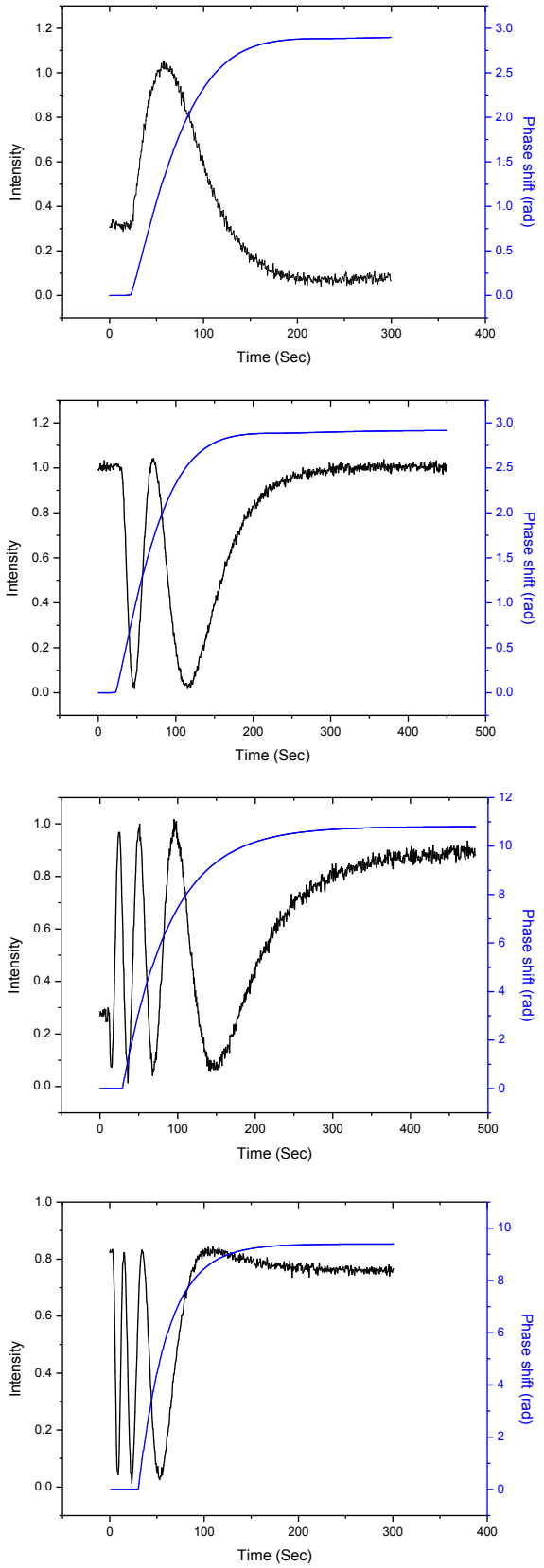


Fig. (7) Antibody immobilization to form the bio-receptor

The bio-sensing results in Fig. (9) shown that the minimal detected concentration of Fumonisin B1 was 0.01 ng/mL, which means this the limit of detection of this setup. The selectivity performance of this system has been carried out by inject other types of mycotoxin; Ochratoxin A (OTA) and aflatoxin B1 (AFB1), these solutions did not produce any oscillation signal even by 1000 ng/mL concentration (Fig. 10), which demonstrated high specific to the targeted molecules.



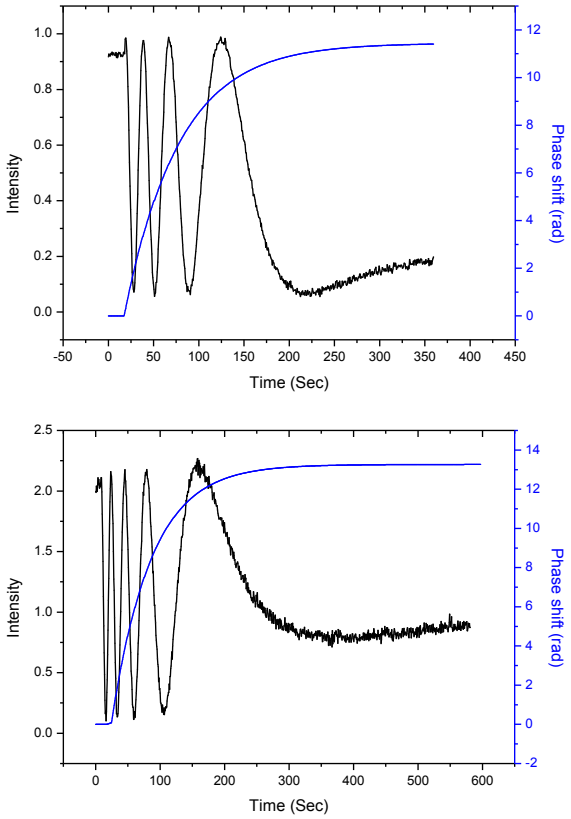


Fig. (8) Corresponding phase shift for injection of Fumonisin B1 in concentrations of 0.01, 0.1, 1, 10, 100 and 1000 ng/mL

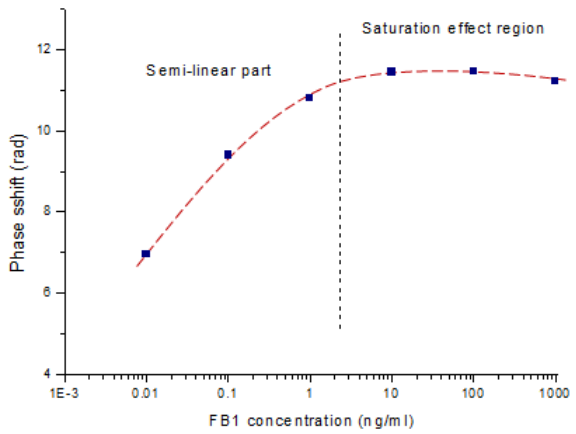


Fig. (9) Phase shifts vs. consecutive injections of Fumonisin B1 concentrations

4. Conclusion

The main objective of this work which was stablish polarization interferometry biosensor using optical planar waveguide for in vitro mycotoxins detection has been achieved. High refractive index sensitivity in rang of 9000 rad/RIU was reached as a result, which is similar to other high performance biosensor methods [24-25], this high optical performance of the system was enabled the system to be good enough sensitive to the targeted biomolecules. A series of biosensing tests

for different concentrations of Fumonisin B1, detection in a direct analysis with specific antibody were successful. The highly sensitivity of the system has been shown through generate a clear enough reasonable signal for the lower concentration (0.01ng/ml) of Fumonisin B1. The corresponding signal was plotted vs concentration of Fumonisin B1, and the incremental built-up of signal has estimated, the calculation show that the LOD was below 0.01 ng/mL.

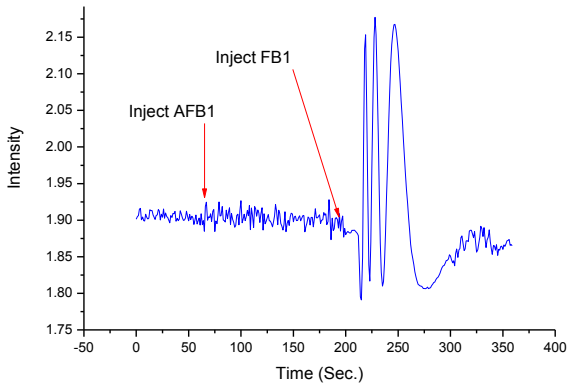


Fig. (10) Selectivity test signal (by inject aflatoxin B1)

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