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Low Weight Bio-Molecular Detection Using Nanostructure Based Localized Surface Plasmon Resonance Sensor

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ABSTRACT

Local surface plasmon set-up based on a Nano shape of metal to stablish high efficiency plasmon resonance. The idea of the proposed system lies in monitoring the wavelength of the resonance. The system has been designed to work in visible light range. The optical sensitivity (RI) of the system has reached 55nm per refractive index unity (RIU. This research present direct detection method of one of the most toxic fungi called aflatoxin). Number of biosensing tests has been conducted to detect aflatoxin B1 in direct immunoassay in the company of specific antibodies which used as bio receptor. The localized surface plasmon resonance sensitivity to a change in the refractive index (RI) has been investigated using different types of nanostructure shapes as a resonance medium. The work was tried to determine plasmon experimental parameters which give rise to achieve best sensing performance. The plasmon resonance spectrum produced by the system and the accuracy in determining and tracking plasmon peak wavelength resonance have allowed to reach detection low concentration of both aflatoxin B1 and Ochratoxin A molecules.

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INTRODUCTION

Traditional technique to quality or quantitative analysis of substances, such as mass spectrometry and chromatography require expensive equipment and professional expertise, making it difficult to meet the growing demand for biological detection and monitoring. Biosensor has emerged as a very suitable alternative to traditional methods due to their advantages that overcome the shortcomings of the previous methods [1-4].

Basically, biosensor is a means to detect the presence of a bio molecules using bio-component to provide physical signal as a product of biological interaction and a transducer element, able to convert this by-product into a proper electronic signal [5-8]. Optical biosensors are the most common biosensor category [9-12]. Optical biosensors possess an important advantage over others analytical techniques as a result of its direct, rapid and label free detection of a lot of chemical and biological substances. Their advantages also involve high sensitivity, cost-effectiveness, small size, and easy to be portable device. Development of the other fields including microelectromechanical systems, microelectronics, biotechnology and molecular biology are contributed to develop and emerge advanced new optical biosensors. Therefore, this technique is constantly evolving. Optical biosensors are mainly employed in the biotechnology industry,

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healthcare, and environmental applications each of them has its specific needs in terms of range of analyte concentration, type of targeted molecules, precision of output and response time [13-17].

Localized surface plasmon resonance has developed as an approach among bio-sensing techniques in that it provides robust, sensitive and facile detection [18-20]. Typical LSPR based bio sensing exploits the correlation of the plasmon resonance with the medium refraction index surrounding a nanoparticle surface [21, 22]. In spite of SPR technologies are widely used, several difficulties remain.

Despite the metal cannot absorb the light by excite the electrons in similar way of the semiconductors, they can show specific light absorption via a resonance between the light waves and metal free electrons. The electric field of light act on surface electrons to move them away from their particles, whereas the coulombic force acts to put it back in its place, so the electrons oscillating under the influence of these two forces in form of stationary waves, called localized surface plasmon resonance [23-25].

LSPR can be described using Mie's solution to Maxwell's equation, occurring as result of movement restriction of electrons in the internal lattice of a metal when the domination of metal structure is scaled-down to a nano level (<100 nm) [26,27].

Eq. 1 shows the correlation of extinction, E(λ), on the shape, density, dimension and surrounded environment of the nanostructure [28-30].

$$E_{(\lambda)} = \frac{24\pi N_A a^3 \varepsilon_m^{3/2}}{\lambda \ln(10)} \left[\frac{\varepsilon_i}{(\varepsilon_r + \chi\varepsilon_m)^2 + \varepsilon_i^2} \right] \quad (1)$$

where N_A is the nanostructure density, a is the nanostructure radius (Nano-structures represented as a sphere), ε_m is the dielectric constant of the medium surrounding the nanostructure (the dielectric constant is assumed to be a positive, real integer and wavelength independent), λ absorbing radiation wavelength, ε_i is the imaginary part of the Nano-structures dielectric function, ε_r is the real part of the Nano-structure's dielectric function and χ is the ratio of the nanostructure.

LSPR spectroscopy can be serving as sensor through transduction the changes in refractive index of the medium that proximity to the surface of the metal nanostructure.

The occurrence of bio recognition events on the metal nanostructure causes change in the refractive index of the medium, which consequently leads to shift on the plasmonic wavelength (λ_{max}) according to Eq. 2:

$$\Delta\lambda_{max} = m\Delta n \left(1 - e^{-\frac{2d}{l_d}}\right) \quad (2)$$

Where, m: represents the bulk RI of Nano-structure, d: represents the thickness of effective adsorbate film and l_d: the length of electromagnetic field decay [31].

There are two main methods to employ this phenomenon for sensing [32].

One of them; utilises the electromagnetic fields which is extending from surface of nanostructure to react and effect on scattered Raman photons, result change in its energy according to the plasmon state. This Raman-LSPR coupling enables plasmon detection through Raman spectroscopy.

The other approach includes monitoring or observed the value of parameters that lead to develop the plasmon resonance, such as wavelengths of light or incident angle, which are associated with and affected by the target substance, such as employed UV-Vis spectroscopy to observe the light wavelength of which induce the plasmon oscillation to happen.

When the metal thin film consists of nano islands (with dimensions less than the wavelength of light) separate from each other, the free conduction electrons at these nano particles surface can be stimulated to oscillate by energy of light wave. This kind of electron- light wave reaction called local surface plasmon, causing strong absorption at specific wavelengths of incident light. This resonance wavelength of this phenomenon is sensitive to the optical properties of the ambient environment, which allow detecting the analyses through monitoring the shift of resonance wavelength when they presented and bind to nanoparticle surface.

Organic molecules usually pass a relatively high refractive index compared to solvent medium, so their presence around nanoparticles results in a detectable redshift in the plasmon resonance wavelength, which makes this mechanism suitable for building a biological sensor [33, 35].

Detecting of small biomolecules especially at low concentration levels is a challenge.

This work aims to develop biosensor based on local surface plasmon phenomenon in terms of sensitivity and limit of detection (LOD). Mycotoxin molecules, specifically ochratoxin (OTA) are the targeted molecules in this work. OTA is a relatively small molecule with the molecular weight of 403.8 Da.

The type of target molecules can be simply changed by changing the type of biological receptor. While maintaining the same performance values for the sensor, so this sensor is considered as free label biosensor.

MATERIALS AND METHODS

Prepare the nanostructure and stablish the bioreceptor

Standard glass slides (75 mm by 25 mm and 1 mm thick) used as platform to stablish the gold Nano structure, were cleaned by immersed them in a hot piranha solution (mixt of H2SO4 and H2O2, 3: 1) for one hour then rinsing by di-ionized water and finally dried up with stream of nitrogen gas.

About 25 nm thickness of gold layer was deposited on the glass slid using thermal evaporator unit (Edwards E306A), this was preceded by the deposition of a 2nm layer of chromium in order to enhance the adhesion of the gold layer at the glass slide. The two stages of deposition were conducted without vacuum breaking which was around 10^{-6} Torr. The flat gold thin film has been converted into Nano pieces (Nano islands) through annealing (de-wetting processes). The annealing temperature around 500 C for two hours was enough to create Nano structure, the result Nanostructure has been checked via SEM image Fig. 1.

To stablish the bioreseptor, antibodies were immobilized on the surface gold islands using immobilization routes inclusive covalent and electrostatic binding Fig. 2. Antibodies (type IgG) at pH 7-8 medium, emergences negative charge, so they can be easy to electrostatically bound to a positively charged layer of hydrochloride (PAH). To avoid the randomly oriented of the antibodies proteins A has been employed as intermediate body, which are electrostatically immobilized first on PAH layer. The IgG molecules bind with protein A by binding site at Fc region of antibody, which make the antibodies oriented vertically Fig. 2. The electrostatic immobilization method for both proteins and antibodies has been proved the successful in our former research, so we used it in this work.

RESULTS AND DISCUSSION

Initially, the structure Nano-islands platform

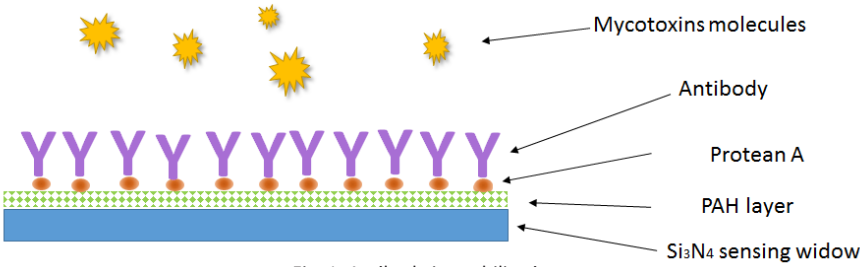

Fig. 1. Antibody immobilization.

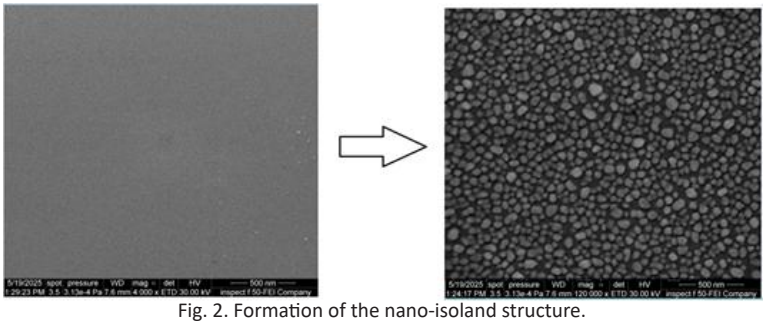

Fig. 2. Formation of the nano-isoland structure.

was formed by exposing the gold thin film to annealing process. Where, it was exposed to a temperature of 400C for 8 hours using electric convection oven. During this process, the flat gold thin film was converted to nanoscale islands. . The samples of nanostructured gold produced were characterized with SEM (FEI-Nova, NanoSEM 200) Fig. 2.

The occurrence of plasmonic resonance was tested by exposing the nanostructure to a wide range of light wavelengths (300-800) nm. The occurrence of plasmonic resonance was tested by exposing the nanostructure to a wide range of light wavelengths (300-800) nm. It was observed that the plasmon resonance peak occurred at a wavelength of 650 nm, as shown in Fig. 3. The selective absorption of wavelength was good evidence to occurrence of the plasmon phenomenon. The optical set up is working as a local surface plasmon system.

The dependence absorbance and sensitivity were investigated by using standard solutions with specific refractive indices as shown in Fig. 4.

The absorbency measurement was performed

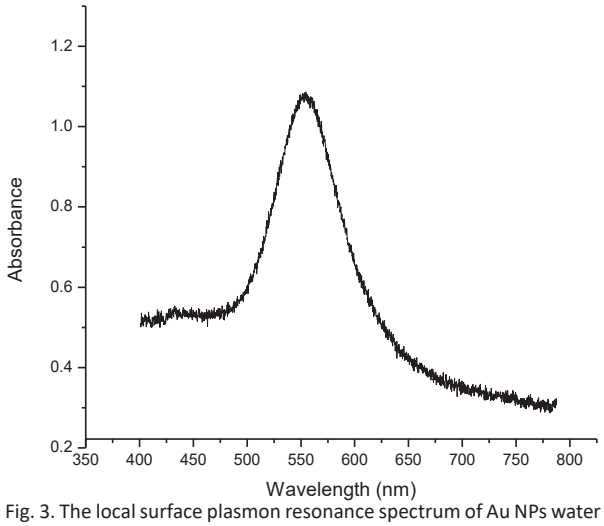

Fig. 3. The local surface plasmon resonance spectrum of Au NPs water using a distilled a second medium.

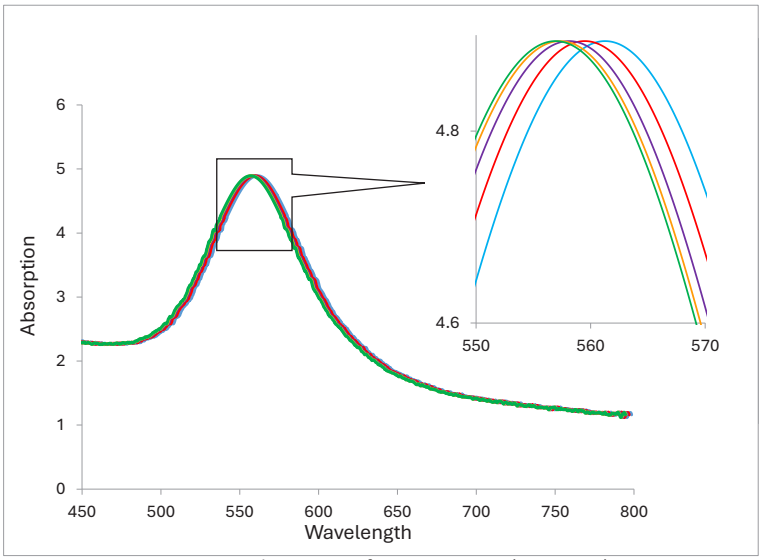

Fig. 4. Optical response of LSPR spectrum (sensitivity).

by measuring the light intensity pass across the Au thin film compared to initial light intensity that reaching the gold nanostructure using Ocean spectrophotometer (FLAME-S-XR1-ES) Fig. 5.

The results of sensitivity test showed a linear response over refractive index range from 1.333 to 1.3480 as shown in Fig. 6. The refractive index sensitivity is commonly reported in wavelength per refractive index unit, for this system was reached, that was a suitable optical performance value for moving forward with the development of bio-sensing.

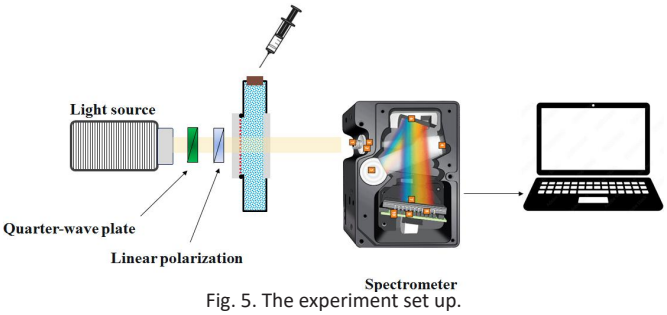

Fig. 5. The experiment set up.

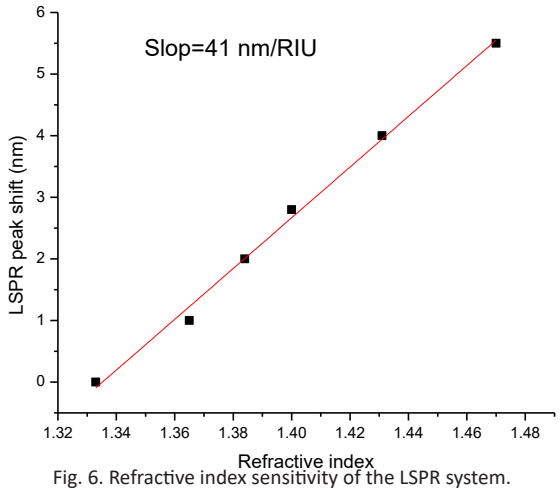

Fig. 6. Refractive index sensitivity of the LSPR system.

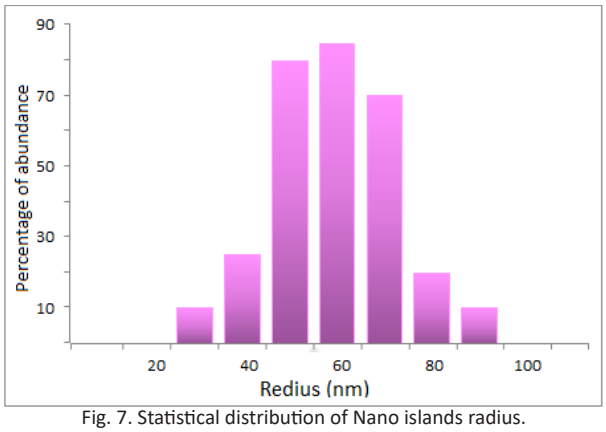

Fig. 7. Statistical distribution of Nano islands radius.

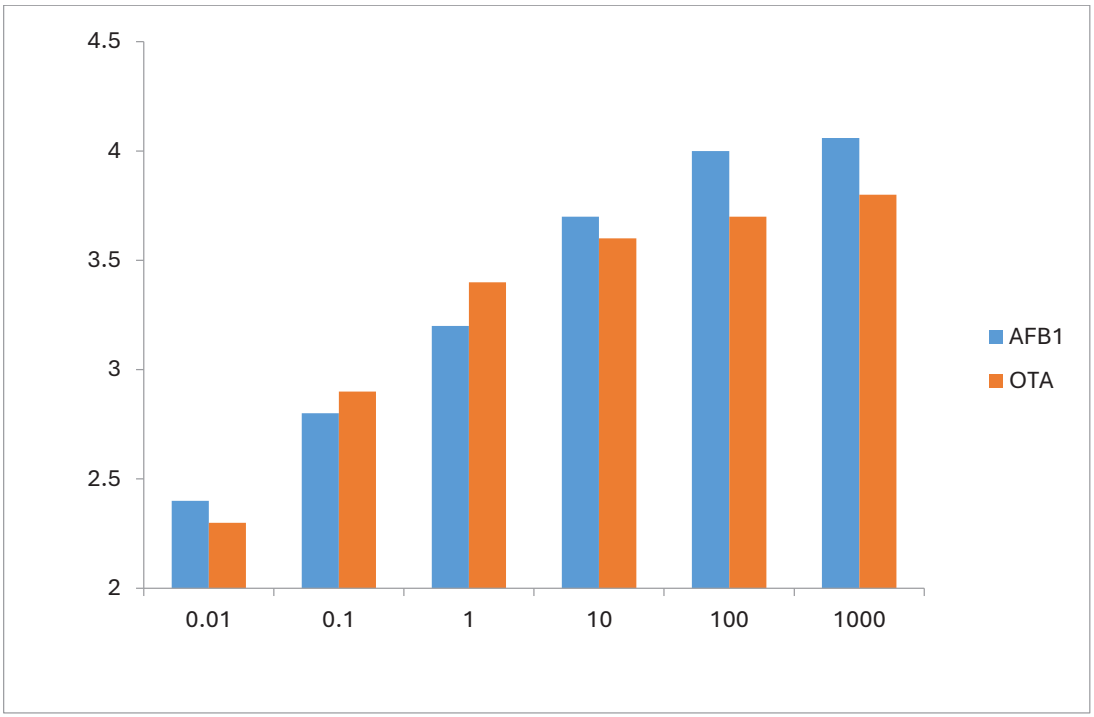

Fig. 8. Bio sensing of Ochratoxin A and Aflatoxin B1 molecules.

The nano-islands structure was tested using SEM, the images show more than eighty percent of them radiuses are around (55 to 85) nm depends on the film thickness. The statistic distribution of sizes for each thickness is approximately eighty percent of the dominant value as shown in Fig. 7.

The performance of the system as a biosensor was tested after preparing the antibody based bio-receptor and attached it to the nano islands layer. Two types of mycotoxins were used for bio sensing testing, Aflatoxin B1 and Ochratoxin A. The system has shown a reasonable signal that exceeded the noise by about three times for the lowest (0.01ng/ml) concentration. The response continued almost linearly until saturation was achieved when the concentration reached (1000 ng/ml) as shown in Fig. 8.

CONCLUSION

In this work, a biosensor system for small bio molecules detection has presented.This research is oriented toward improve the bio sensing based on develop the nanostructure of the bio receptor. The influence of the gold nano islands structured on the sensitivity enhancement has been studied. The theoretical explanation of the system was assisted to identify which direction should be given to reach the aim.

The performance of the system has been evaluated as low concentration biomolecules detection. The plasmon resonance spectrum produced by the system and the accuracy in determining and tracking plasmon peak wavelength resonance have allowed to reach detection low concentration of both aflatoxin B1 and Ochratoxin A molecules.

Finally, the effect of nanostructure on the biosensing has been demonstrated, allowing to more investigation and development based on nanostructure control.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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