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Sensitive optical biosensor based on surface plasmon resonance using ZnO/Au bilayered structure

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ABSTRACT

The present article represents the preparation of highly sensitive optical biosensor using an indigenously assembled table top Surface plasmon resonance (SPR) technique. Surface plasmon modes have been excited at the ZnO-gold interface exploiting Kretschmann configuration. RF-magnetron sputtering has been utilised to deposit ZnO thin films further used for the immobilization of specific enzyme GOx by physical adsorption technique. The SPR reflectance curves were recorded for the GOx/ZnO/Au/prism system in angular interrogation mode in Phosphate buffer saline (PBS) solution by varying analyte (glucose) concentration from 0 mg/dl to 300 mg/dl. The transient variation in the intensity of reflectance (i.e. response) at fixed SPR resonance angle for increasing concentration of glucose is also reported. The prepared sensor exhibits enhanced response (0.75 for 25 mg/dl) with high specificity and good linearity beyond the physiological range indicating the realization of an efficient optical biosensor.

1. Introduction

Enormous efforts have been done in the field of chemical and biochemical sensing using surface plasmon resonance (SPR) technique [1–4]. Various biological compounds including proteins, enzymes, pesticides for affinity analysis, monitoring of real time biomolecular reaction etc. has been detected using SPR technique [5–10]. Surface plasmons (SP) can be considered as the electromagnetic excitations at the interface of metal-dielectric which are transverse magnetic (TM) in nature with their amplitude exponentially decaying in metal and dielectric medium. The SPs are excited by the evanescent wave (TM polarized). To excite the SPs, Kretschmann configuration is the most preferred in which a metal layer of optimized thickness is directly deposited on the glass prism which is used to excite the SPW [11]. Now, resonance occurs if the surface plasmon wave (SPW) propagation constant becomes equal to the evanescent wave propagation constant indicating a minimum in the reflection at resonance angle. Therefore, SPR can be considered as the resonant excitation of surface plasmons due to the generation of evanescent wave by total internal reflection of the light wave propagating at the interface of prim-metal interface [12]. The resonant conditions are dependent on the sensing medium dielectric constant (or refractive index). The two approaches for studying SPR are: wavelength interrogation and angular interrogation method. In wavelength inter-

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rogation method, angle of incidence is kept constant and wavelength of light is changed. In angular interrogation mode, angle of incidence is a variable quantity keeping wavelength of light fixed. The refractive index of the sensing medium is determined using the value of resonance parameter. The SPR technique provides the advantage of real-time monitoring of the interaction of biomolecule (antibody or enzyme) which is immobilized on the sensing surface with its specific analyte by examining the refractive index changes occurring at the interface. The sensing principle of SPR technique is the change in refractive index at the interface of metal-dielectric on exposure with the sensing material [13]. Also, sensing by SPR is advantageous because of it being room temperature operating and simple detection technique [14,15].

The glucose sensing based on SPR technique using glucose oxidase enzyme (glucose specific) is very specific and highly sensitive to glucose concentration. There is no interference from constituents found in blood. Glucose sensing is very crucial in the control and diagnose of diabetes [16]. Therefore, frequent monitoring of glucose level is very important to prevent any complications associated with diabetes and hence a simple room temperature operated sensor is required to meet this goal [17]. There are various reports on glucose sensing and some of them are available in commercial market also based on several techniques such as electrochemical signal transduction (EST) [18], fluorescence signal transmission (FST) etc. [19]. Out of all the techniques, SPR has emerged as a promising technique for the ascertainment of various biomolecules in recent years [20]. This technique offers a promising alternative to other conventional techniques due to the advantage of real-time monitoring and allows rapid, sensitive and label-free detection [21]. SPR based biosensors have been explored tremendously and there are various reports available in literature exploiting SPR technique for biosensing applications utilizing the commercial setups available on SPR technique [22,23]. Mostly commercial setups are available for biomolecule detection having high sensitivity but limited range of angle of incidence. They are bulky, expensive and the chip used for measurements is one time usable. Also, they work at fixed wavelength of incident light having less refractive index range studies. The dielectric constant of any material can be estimated using the present setup. The present setup uses SPR technique for versatile applications, low cost and user friendly, provides the facility for measuring the SPR reflectance curve at different wavelengths, study the material property with varying physical parameters (T, B, etc.) and studying the optical properties of dielectrics including bio-molecules in solid, liquid, or gaseous media. In the present article, an attempt has been made to exploit this indigenously developed SPR setup for the development of glucose biosensor and the results obtained are discussed in detail.

The most crucial and important aspect in designing a highly sensitive and efficient biosensor includes the selection of an appropriate matrix for supporting the successful immobilization of the biomolecules such that the stability and biofunctionality of the biomolecule are maintained. There are several matrices available in literature for the proper immobilization of glucose oxidase (GOx) enzyme to realize an efficient glucose sensor such as metal nanoparticles (NPs), metal oxides and polymers etc. Amongst them, ZnO arose as an appropriate matrix for successful attachment of biomolecules [24]. ZnO finds diverse applications in the field of optoelectronics, photovoltaics, sensors, data storage, biochemical/chemical sensors etc [25,26]. Zinc oxide (ZnO) is a semiconductor having wide bandgap of approximately 3.4 eV and possesses an isoelectric point i.e. IEP of ZnO = 9.5 making it appropriate for attachment of proteins having low IEP (glucose oxidase having IEP approximately equal to 4.22) by physical adsorption technique. It also exhibits good electron communication capability and high catalytic efficiency. Diverse kinds of biomolecules have been reported to be immobilized on the surface of ZnO thin film for the realization of specific biosensors. Therefore, ZnO as a dielectric layer could be exploited for the determination of dielectric properties of the biomolecules immobilized on its surface using surface plasmon resonance technique. Therefore, ZnO has been identified as the promising matrix for the immobilization of urease and detecting urea efficiently.

2. Experimental details

In this work, laboratory assembled SPR measurement system is used to excite SP mode in Kretschmann configuration in which, a metal film (Au thin film) of optimized thickness 40 nm deposited directly on one face (i.e. hypotenuse face) of the glass prism (BK-7) by the technique of thermal evaporation. Au thin film thickness is monitored using a quartz crystal thickness monitor. Here, 200 nm thick ZnO film corresponding to sharp SPR reflectance spectra is deposited on Au coated prism using rf magnetron sputtering technique utilizing a 2″ diameter target of Zn metal (99.999% pure). There are various reasons for the choice of rf-magnetron sputtering such as (i) high deposition rates, (ii) easy sputtering of any material like metal, alloy or compound, (iii) depositing highly pure films etc. The rf magnetron sputtered ZnO thin films are deposited under the gas mixture of 60% Ar and 40% O_2 at the deposition pressure of 20 mTorr and a in situ annealing temperature of 250 °C. The freshly prepared 1 mg/ml solution of GOx enzyme in Phosphate Buffer Saline (PBS) solution is used for the immobilization of enzyme on the surface of ZnO film via strong electrostatic interaction to retain the bio functionality of the biomolecules [27,28]. The explanation of the SPR measurement system is given in our previous work [29]. The SPR curve for buffer/biomolecules/ZnO/Au/prism system in angular interrogation mode was measured in the PBS buffer solution. A liquid sample cell indigenously designed for aqueous media was attached to the prism where there is feasibility that the face of the prism loaded with immobilized enzyme is in direct exposure with the aqueous medium as shown in Fig. 1 where 'i' is the angle of incidence (external) and 'θ' is the angle of incidence (internal). Here, SPR reflectance curve is plotted between reflectance vs. θ (internal angle of incidence). SPR measurements were also carried out in static mode in which the variation of SPR resonance angle with an increase in concentration of glucose is recorded as well as dynamic mode where the variation in the minimum reflectance with an increase in glucose concentration at fixed resonance angle is measured with time using a CCD camera.