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Glucose oxidase immobilized PANI cladding modified fiber optic intrinsic biosensor for detection of glucose

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ABSTRACT

In the present study, we report design, development and study of polyaniline (PANI) cladding modified fiber optic intrinsic glucose biosensor (FOIGB) for glucose sensing applications. Chemically and biologically sensitive material PANI has been exploited for the preparation of sensing element by cladding modification technique. Enzyme-glucose oxidase (GOx) was immobilized on porous supportive matrix of PANI using glutaraldehyde as a cross-linking agent. As-synthesized and deposited PANI film on fiber optic core was characterized by ultraviolet–visible and Fourier transform infra-red spectroscopies. X-ray diffraction and field emission scanning electron microscopy were employed to study structure and morphology of modified cladding. Sensing parameters, such as modal power distribution (MPD), sensing response, selectivity and stability were analyzed in order to study the performance of sensor. These parameters were determined by measuring change in optical power and MPD. The sensor, stored at a temperature 4 °C, showed lowest detection limit of 10 nM and found stable up to 36 days. It also showed good selectivity toward glucose and stability with high result reproducibility.

1. Introduction

As per the new study [1], there will be largest number of diabetic patients in India, China and USA by 2030, and it has been predicted that every fifth person will be the Indian. Due to diabetes, major healthcare problems have been increased with lot of complications, which lead to increase in morbidity and mortality. In addition, diabetes is linked too much with the heart diseases and stroke in this aspect [2–4]. This dangerous disease is caused due to unbalance of sugar in blood, which results in a variety of knottiness. Therefore, for the prevention of such complicated diseases exact care needs to be taken [5]. In order to monitor glucose level in blood, first glucose sensor was developed by Clark in 1956 [6] and later on by Clark and Lyons in 1962 [7,8]. The developed sensors were based on electrochemical analysis of blood glucose. Electrochemical analysis of blood glucose level had been the method of choice for decades but increasing demand for monitoring devices promoted researcher to search new type of methods for the glucose detection. In addition to electrochemical (amperometric, voltametric and potentiometric) sensors, other types of sensors, such as conductometric, calorimetric, mechanical (piezoelectric) and optical sensors have been proposed and now widely employed for the determination of glucose in human blood [9–15]. Recently, use of optical fibers in the design and development of fiber optic biosensor has received considerable interest as compared to other traditional sensors due to their small and compact size, high sensitivity and reliability, fast response, ability to be multiplexed, remote sensing capability, ability to be embedded into textile structures, immunity to electromagnetic interference, non-conducting and intrinsically safe for patients [16–20]. Traditionally, optical fiber sensors were used to measure physical parameters such as temperature and pressure [21]. Now days, they are also being used to sense the chemical and biological species owing to capability of the optical fibers to modulate properties of propagating light such as intensity, phase and/or polarization [22,23].

Portaccio et al. [24] have prepared cladding modified multimode optical fiber biosensor via sol–gel entrapping of GOx as sensing element coupled to a fiber optic transducer. Lin et al. [25] reported glucose fiber sensor integrated with heterodyne interferometry and measured the phase difference arising from the chemical reaction between glucose and GOx. However, sol–gel entrapping and heterodyne interferometry processes are not convenient and versatile due to leaching problem and cannot be

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repeatedly used. The use of cross-linking immobilization method has been proved a good choice to overcome the leaching problem [26–28]. The process of immobilization of GOx with cross-linking via glutaraldehyde on polymeric matrix has been utilized in conjunction with electrochemical methods but the combination suffers from complicated and expensive experimental arrangement and nonreproducible results [26,29–31]. The cladding modified fiber optic intrinsic biosensor (FOIB) [32], which uses a simple photo detector for sensing purpose, has considerable advantages over electrochemical sensors [9]. However, it is essential to continue the research in this field with new materials and approach so that the sensitivity and stability of the sensors can be improved.

In the present study, we have fabricated cladding modified fiber optic intrinsic glucose biosensor (FOIGB) by modifying portion of the cladding of multimode optical fiber with a PANI polymer matrix and immobilized GOx by cross-linking via glutaraldehyde. The sensing response toward glucose in solution with concentration ranging from 10 nM to 100 mM has been analyzed. The stability of cladding modified FOIGB has been estimated by studying response of same FOIGB for 50 days with an interval of 2 days. The developed FOIGB showed no considerable deviation in the sensitivity up to 36 days. The modal power distribution (MPD) measurement has been employed to confirm the coupling of higher optical modes to the core that is responsible for increase in optical power at the output. The selectivity was studied by comparing response of sensor toward glucose, and other interfering species like urea, ascorbic acid, lactic acid, fructose and sucrose. Prepared PANI polymer matrix was characterized by using various characterization techniques, such as ultraviolet–visible (UV–vis) spectroscopy, X-ray diffraction (XRD) analysis, Fourier transform infrared (FT-IR) spectroscopy, field emission scanning electron microscopy (FE-SEM) and optical microscopy. The results have been presented.

2. Experimental

2.1. Materials and methods

Aniline (monomer) and ferric chloride (oxidant) were purchased from Fisher Scientific, USA for the synthesis of PANI. Glucose oxidase (GOx, *Aspergillus Niger* extra pure, 125 units/mg, 1 unit is capable to oxidize 1 μM of D-glucose to D-gluconolactone and H_2O_2 per min at pH 7.0 at 25 °C) was procured from Sisco Research Laboratories (SRL), India. Analyte glucose, glutaraldehyde solution (25%), urea, ascorbic acid, lactic acid, potassium dihydrogen orthophosphate, sodium hydroxide, and acetone were purchased from SD Fine chemicals, India. Fructose and sucrose were obtained from Loba Chemie, India and Merck, Germany respectively. All the synthesis processes were carried out in freshly prepared double distilled water and in phosphate buffer (pH 7.4). All the chemicals were of analytical grade and used as received without further purification.

In a typical procedure, the stock solutions of GOx in proportion 2 mg/ml and glucose were prepared in 0.1 M phosphate buffer of pH 7.4 and kept at temperatures 4 and 10 °C, respectively for 24 h. The optimum value of pH was chosen 7.4 because human blood has the pH 7.4 [33].

2.2. Preparation of sensing element

About half meter long plastic cladded silica core multimode optical fiber of core/cladding dimensions 425/320 μm was taken to prepare PANI cladding modified FOIGB. Both ends of the optical fiber were polished and connected to the SMA905 connectors to couple laser beam at input and charge coupled device (CCD) camera beam profiler at the output. The ends of the optical fiber were

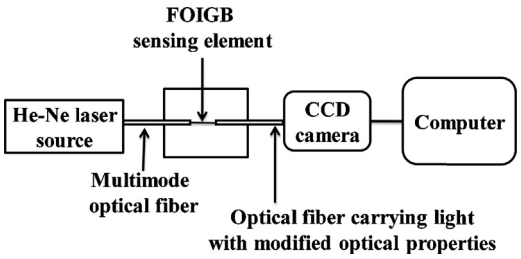

Fig. 1. Block diagram of FOIGB setup.

cut with the help of stripper and then cleaved on very fine silicon carbide paper followed polishing by fine polish paper until it showed clear modes by integrating light at its one end. The original cladding of optical fiber of 2 cm portion was removed mechanically with the help of stripper and surgical blade. The removed surface was cleaned several times with acetone and double distilled water before modification. PANI cladding modified FOIGB was prepared by depositing a layer of PANI on cladding removed portion by chemical polymerization method [34] at a room temperature with reaction period of 12 min. In a conical flask, 0.2 M aqueous solution of aniline monomer was taken and 0.05 M $FeCl_3$ solution was added drop by drop with a constant stirring. The cladding removed sensing portion was submerged in it during the polymerization to deposit a thin layer of PANI as an active cladding. After deposition, sensing element was firstly dried for 1 h at room temperature and then washed 2–3 times with double distilled water. The solution of enzyme-GOx prepared in 0.1 M phosphate buffer of pH 7.4 was immobilized on sensing element by cross-linking via glutaraldehyde [35,36]. Sensing element was dried for 30 min at room temperature and washed 2–3 times with phosphate buffer solution before it was used for sensing of glucose. The entire experiment was performed in dark room and the sensing chamber was also enclosed in another chamber for protected from any stray light.

2.3. Characterizations

The UV–vis spectrum of as-synthesized PANI film was recorded using portable UV–vis fiber optic spectrophotometer (BLK-C-SR, StellarNet, USA) in the spectral range 200–1000 nm. The structural data was acquired from powder XRD using X-ray diffractometer (MiniFlex II, Rigaku, Japan) with CuK_α radiations of wavelength $\lambda = 1.5406$ Å. The surface morphology and the size of PANI nanopores were observed using field emission scanning electron microscope (FE-SEM) (S-4800, Hitachi, Japan). The functional groups are confirmed by using FT-IR spectrum recorded using IR double beam spectrophotometer (8400S, Shimadzu, Japan). The measurement of variation in power and MPD were carried out using a set up as shown in Fig. 1 consisting of CCD camera beam profiler (BC106-VIS, Thorlabs, USA), He–Ne laser (λ – 632.8 nm, power – 2 mW) and power meter.

3. Results and discussion

3.1. Optical studies of PANI

Fig. 2 reveals the UV–vis spectrum of as-synthesized PANI film deposited on fiber optic core. It is sensitive tool to study the electronic structure and elucidation of the polymer chain of PANI. The emraldine form of PANI show two absorption peaks at ~272 and ~732 nm. The peak at ~272 nm may be due to $\pi-\pi^*$ transitions and a broad absorption peak at ~732 nm is attributed to the transition between benzenoid–quinoid ring and conducting nature of PANI film [37]. Fig. 3 elucidates the FT-IR spectrum of PANI along with its functional group frequencies. The spectrum shows all the expected