



Pani/Zno Modified Fiber Optic Intrinsic Biosensors for Detection Of Glucose-Analyte

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Abstract:

In this research paper, brief idea of biosensors, their importance and applicability are presented. The biosensors are discussed with the special applications in detection of glucose-analyte. In present work, cladding modification technique used for the fabrication of cladding modified fiber optic biosensor. The prepared sensors were studied by detecting the parameters such as absorption-sensitivity and selectivity in the ultraviolet-visible (UV-vis) spectral range. The characterization techniques used for the sensor confirmation includes UV-vis spectroscopy, X-ray diffraction (XRD), and optical microscopy.

Keywords: Biosensor; Fiber optic; Cladding modification; Glucose; PANI; ZnO.

Introduction:

Diabetes mellitus is a worldwide public health problem. The complications of battling diabetes are numerous, including higher risks of heart disease, kidney failure, blindness or finally death. The diagnosis and management of diabetes mellitus thus requires a tight monitoring of blood glucose levels. However, glucose is the most commonly tested analyte by millions of diabetics and literally glucose biosensors account for about 85% of the entire biosensor market. Such huge market size makes diabetes a model disease for developing new bio-sensing.

Seventy years have been passed to design first glucose sensor by Clark in 1956 and Clark and Lyons in 1962 based on enzyme electrode in order to monitor

glucose level in blood. In addition to diabetes control, such devices offer great promise for other important applications, ranging from food analysis to bioprocess monitoring. A variety of approaches have been explored in the operation of glucose enzyme electrodes. Many methods are available for the determination of glucose in the human blood, such as electrochemical (ampere-metric, volta-metric, potentiometric), conducto-metric, calorimetric, mechanical (piezoelectric), and optical (fluorescence, absorption, phosphorescence, chemi-luminescence, etc.) [1-4].

Presently, the use of optical fibers in the design and development of fiber optic sensors has received considerable interest as compared to traditional electrode based sensors because of small and compact size,

sensitivity, reliability, fast ability to be multiplexed, remote sensing ability to be embedded into textile structures, immunity to electromagnetic interference, non-conducting and intrinsically safe for patients. Fiber optic sensors include fiber with Bragg gratings, modified claddings, tapered end, micro or macro bends and fiber-optic coupler sensors [5-7].

The cladding modification is achieved by removing a small portion of the cladding of optical fiber and replacing it with an active cladding. Generally, a polymer matrix provides porous and biocompatible matrix for the immobilization of enzymes [8-11]. To further improve the performance of the biosensor, other organic/inorganic materials or nano-materials could be introduced into PANI to improve stability, sensitivity, selectivity and efficiency of the sensor. For glucose detection; enzyme-GOx is utilized as a biological recognition element. The enzyme-GOx catalyzes glucose into gluconolactone and H_2O_2 in the presence of O_2 , which induce some kinds of signals, which can be picked up and interpreted. Optical fiber biosensors can be used in combination with different types of spectroscopic technique, e.g. absorption, fluorescence, phosphorescence, Raman, surface plasmon resonance (SPR), etc. When absorbance is measured the biological receptor can be immobilized close to the optical fiber or directly on its surface [12-17].

In the present research paper, preparation, characterization and result of the fiber optic biosensors has been studied. Highlight on the components used for the fabrication of fiber optic sensors i.e. Optical fiber, source and detector etc. has been given. For the cladding modification of sensor PANI and PANI-ZnO nanocomposite material was used. Enzymes GOx was immobilized on polymeric matrix by cross-linking via glutaraldehyde on it. The sensing responses toward glucose in solution with

2.5 nM and 10 μ M have been analyzed. Moreover, the selectivity of the sensors was checked towards glucose by comparing with urea solution. Prepared polymer matrices were characterized using various characterization techniques, such as ultraviolet-visible (UV-vis) spectroscopy, X-ray diffraction (XRD) analysis and optical microscopy.

Experimental

Material 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 1M of d-glucose to d-gluconolactone at pH 7.0 at 25°C) was procured from Sisco Research Laboratories (SRL), India. Analyte zinc chloride, sodium hydroxide palletes, glucose, glutaraldehyde solution (25%), were purchased from sd fineCHEM, India. The entire synthesis processes were carried out in freshly prepared double distilled water and in 0.1M phosphate buffer solution (pH 7.4). All the chemicals used were of analytical grade.

Optical Setup:

Fiber optic biosensor setup for glucose detection is as shown in Fig. 1. One end of sensor was fixed between the optical source (Halogen-Deuterium lamp) and other was coupled to the UV-vis spectrophotometer (BLK CSR StellarNet, USA).

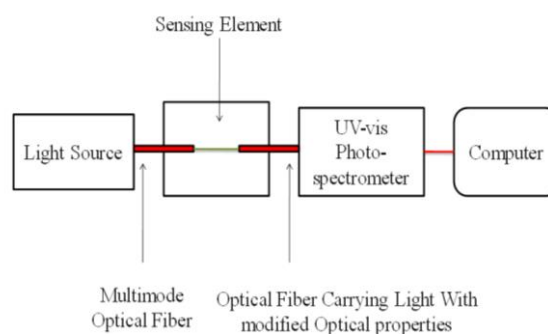


Fig.1: Fiber optic sensor assembly

The straight region of the fiber will held in an indigenously developed sensing cell for detection purpose. When the glucose solution was added in the cell, light emerging from the other end of the fiber was collected by the spectrophotometer and the absorption spectra for the various concentrations of glucose was recorded using indigenously developed computerized setup. The same assembly was used to determine the selectivity toward glucose.

Preparation of Optical Fiber Sensing Probe:

In the present experiment arrangement, the preparations of FOIGBs have been done using cladding modification technique. It is possible to develop sensing region on optical fiber for sensing purpose. In the sensing region, an evanescent wave interacts with the analyte and produces a signal by changing its optical properties. The cladding modification can be done at a desired portion of optical fiber and involves two major steps-

- a) Removal of the passive cladding from the optical fiber and
- b) Application of an active cladding as an absorbing medium on the core.

The FOIGBs were prepared using half-meter long piece of multimode plastic clad silica (PCS) optical fiber of 750 μm diameter (450/300 μm core/cladding). The ends of the optical fibers were cut with the help of stripper and surgical blade. Both the ends were cleaved and polished by using silicon carbide and very fine polish papers of 1200 (5 μm) and 0.3 μm roughnesses in the respective order to enhance the coupling of light in the fiber. Those ends then connected to the SMA905 connectors with the help of an adhesive to couple light beam at input and UV-vis spectrophotometer at the output for detection.

FOIGB sensing element is fitted in a cell to interact with solutions. Care was

taken, while preparing a sensing element cell that the sensing element should be completely dipped into the solution to interact with analyte solutions. All the measurements were carried out at constant temperature of 27°C.

Preparation of GOx and glucose solutions:

In a typical procedure, the stock solutions of GOx were prepared in 0.1M phosphate buffer solution of pH 7.4 and kept at temperature 4°C for 24 h before use.

Immobilization of enzyme-GOx:

On the cladding modified thin layer of polymer matrices the enzyme-GOx was deposited using immobilization process to prepare the FOIGBs. Before immobilization of GOx, the sensing element was rinsed with phosphate buffer solution. The GOx was immobilized over modified polymer matrix surface through cross-linking technique via 1% glutaraldehyde solution diluted in phosphate buffer using layer-by-layer technique with the help of an aid-syringe. The sensing elements were then allowed to dry for 30 min and washed 2-3 times with phosphate buffer solution. Phosphate buffer also helps to prevent the enzyme-GOx denaturation [1-5].

Result and Discussion:

UV-vis Spectroscopy:

The UV-vis spectrum of as-synthesized PANI and PANI doped ZnO deposited on fiber optic core which is shown in Figure 2. The PANI spectrum shows two peaks. The peaks are attributed to $\pi \rightarrow \pi^*$ transition in the benzenoid rings and $\pi \rightarrow \pi^*$ transition in quinoid rings of polyaniline. (1) In the case of PANI-ZnO nanocomposite, the peak is ascribed to the selective interaction between ZnO and quinoid ring of polyaniline.

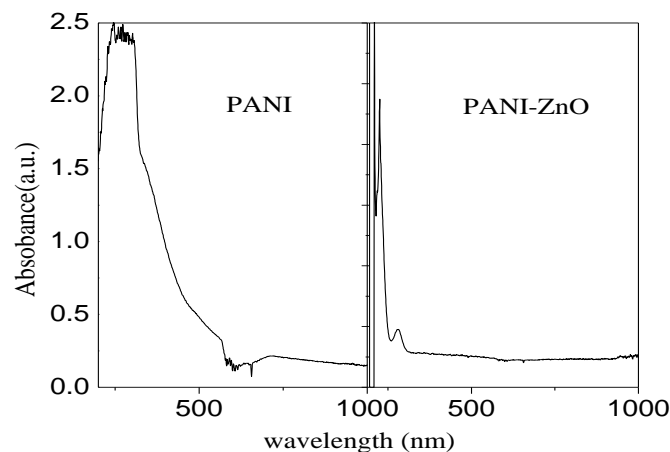


Fig. 2: UV-vis spectrum of PANI and PANI-ZnO nanocomposite.

XRD Study:

Figure 3 shows X-ray diffraction patterns of PANI and PANI-ZnO nanocomposites. The presence of the rigid aromatic backbone of PANI makes it a semi-crystalline polymer. Also, it can be seen that the XRD patterns of nanocomposites are

similar to that of PANI since the presence of insignificant content of ZnO nanorods. The X-ray diffraction patterns of PANI-ZnO nanocomposites confirmed formation of the conducting organic-inorganic nanocomposites.

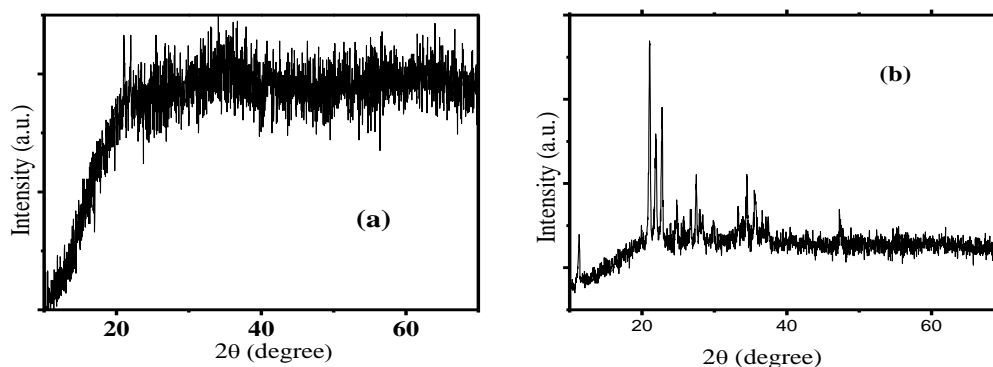


Fig.3: XRD pattern of (a) PANI and (b) PANI-ZnO nanocomposite thin film.

Optical microscopy:

Figure 4 shows optical images of FOIGB sensing element captured by optical microscope (AxioCam, Germany) equipped

with high-resolution digital camera for the confirmation of cladding modification and measurement of film thickness of modified cladding.

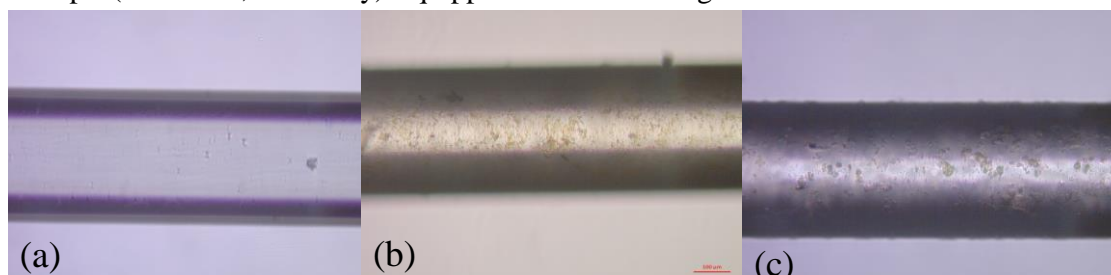


Fig.4: Optical images of (a) core and cladding modified with (b) PANI and (c) PANI-ZnO nanocomposite materials.

Sensing response study:**For Pani-Foigb:**

In the present project, 2.5nM–10 μ M glucose solutions were used to check the sensing response. The FOIGB was excited by off-axis illumination with He–Ne laser. In the sensing experiment, the sensing element was fixed in indigenously prepared sensing chamber and solutions of glucose with various concentrations were added one by one in the chamber. The sensor response

in the form of change in the output power was recorded. Figure 5 depicts the response of prepared FOIGB in terms of variation in power measured after regular interval of time for various concentrations of glucose solutions. The power increases, in detectable limit, with increase in concentration of glucose solution. It reveals that the present biosensor exhibits excellent response to glucose concentration from 2.5nM to 10 μ M.

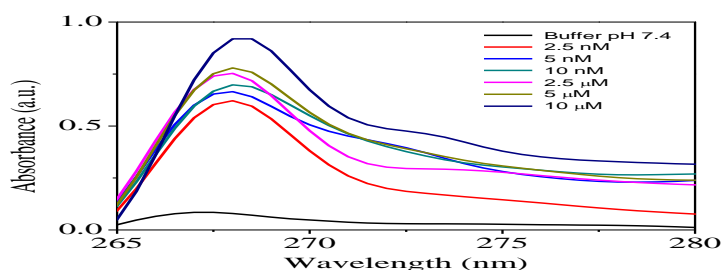


Fig.5: Sensing response for PANI cladding modified FOIGB.

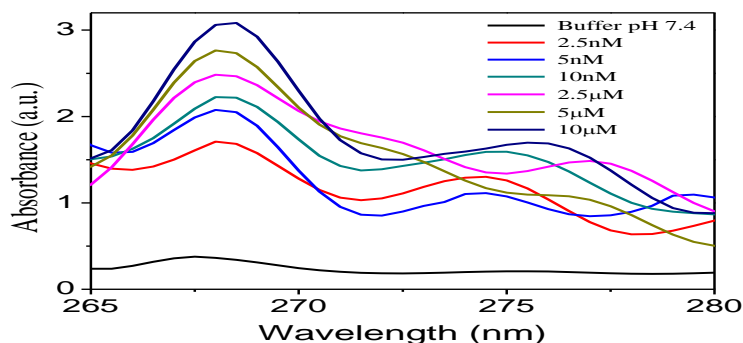
For PANI-ZnO nanocomposite FOIGB:

Fig.6: Sensing response for PANI-ZnO nanocomposite cladding modified FOIGB

In the present paper also PANI-ZnO, 2.5nM–10 μ M glucose solutions were used to check the sensing response. The power increases, in detectable limit, with increase in concentration of glucose solution.

Selectivity:

The selectivity of the FOIGB toward glucose analyte was confirmed by comparing response of FOIGB toward 0.1M phosphate buffer, glucose and urea solutions. All the solutions (glucose and urea) were prepared in 0.1M phosphate buffer. Figure 7 shows the absorbance spectra to determine the selectivity of

FOIGB towards glucose. The urea solution has not shown any absorbance band. In addition, it has not shown any considerable change as compared to the buffer solution. The slight increase in absorbance may be because of variation in refractive index of surrounding solution in presence of urea in the buffer solution. But the variation in absorbance is very large for the glucose solution.

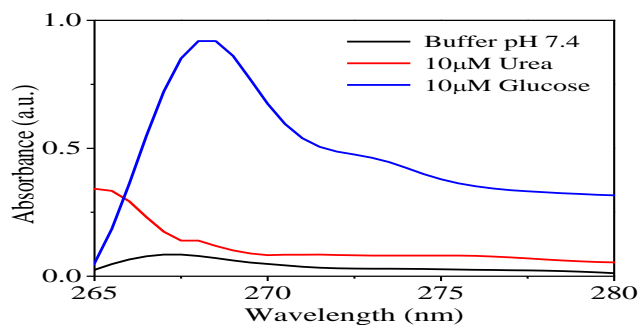


Fig.7: Selectivity of FOIGB towards glucose

Conclusions:

The PANI cladding modified fiber optics intrinsic biosensor for detection of glucose has been reported in this work. The stability and linearity of the fiber optics glucose biosensor have been studied under the same condition after repeatable use, and it was worked very well, as kept at 4 °C. In addition, UV- visible, XRD, and Optical microscopy of thin film have been studied to confirm the formation of conducting polymer over the sensing portion. The developed FOIGB may be useful for the practice purpose, it show very fast response. Glucose biosensors have evolved to be more reliable, rapid, and accurate and are also more compact and easy to use. PANI provides higher specific surface for immobilization of enzymes. Thus, the biosensor performance was effectively improved. All the results show that PANI can be effectively used as promising material for the biosensor designs and other biological applications as confirmed from the UV-vis, XRD and optical microscopy.

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