Journal of Advances in Applied Sciences and Technology

ISSN : 2393-8188 (print)
2393-8196 (online)
www.milliyasrcollege.org.journal.php

CLADDING MODIFICATION WITH PANI-SULPHANILIC ACID COMPOSITE FOR FIBER OPTIC INTRINSIC GLUCOSE BIOSENSOR

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ABSTRACT:
The study of fiber optic intrinsic glucose biosensor (FOIGB) with modified cladding by polyaniline-sulphanilic acid (PANI-S) composite for the glucose detection has been presented. A cladding of as-prepared PANI-S composite was used for the immobilization of enzyme glucose oxidase (GOx) via glutaraldehyde.The sensor exhibits excellent variation in power with the glucose concentration. PANI-S composite material was able to provide a suitable polymer matrix for immobilization of GOx for the preparation of sensor. The PANI-S composite has been characterized using ultraviolet-visible spectroscopy, X-ray diffraction, and optical microscopy. The sensing response of FOIGB was studied for various concentrations of glucose by measuring optical power.

***KEYWORDS*:** *Fiber optic, Biosensor, Cladding modification, Immobilization, Cross-linking.*

1. INTRODUCTION

In 1962, Clark and Lyon was developed the first biosensor for glucose detection [1]. After that biosensors have been intensively studied and extensively utilized in various fields. A biosensor can measured signals electrochemically, optically, acoustically, mechanically, calorimetrically, or electronically, which can be proportional to the concentration of the analytes [2]. As per the utility glucose, biosensors have been applied to a wide variety of analytical problems in medicine, drug discovery, the environment, food, security and defence. In the mentioned fields, the glucose determination is very important for the diagnosis of diabetes. [3, 4] Glucose can be determined using several biosensing techniques based on the enzymatic reaction of glucose oxidase (GOx) with glucose. However, most of the glucose sensors have easy to be affected by electronic or magnetic field [5]. The fiber optic sensors have the advantages over electrochemical and other sensors that include miniaturization, high accuracy, immunity from the disturbance of electric and magnetic fields, fast response, low cost, geometric flexibility and the lack of electrical connection between the sensor and the sample [6, 7]. Thus more and more attention has been paid on the research of fiber optic biosensors around the world. The immobilization of enzymes on optical fiber plays an important role in the fabrication of fiber optic biosensors. It can be possible by cladding modification approach on removing a small portion of the cladding of optical fiber and replacing it with an active cladding of polymer [8]. For the cladding modification, PANI found an intelligent material among various polymers due to its unique properties. It has suitable accommodating surface behaviour for immobilization of biomolecules. Moreover, organic/inorganic materials could be introduced into the PANI matrix provides a suitable matrix for the maximum immobilization of enzymes [9]. Enzymes could be immobilized on polymeric matrix using traditional methods

include adsorption, encapsulation, entrapment, covalent binding, and cross-linking [10].

In the present study, we have fabricated cladding modified FOIGB by modifying portion of the cladding of multimode optical fiber with a polyanliline-sulphanilic (PANI-S) acid composite polymer matrix and immobilized GOx by cross-linking via glutaraldehyde on it. The sensing responses toward glucose in solution with 10 and 100 mM have been analyzed. Prepared PANI-S composite polymer matrix was characterized by using various characterization techniques, such as ultraviolet-visible (UV-vis) spectroscopy, X-ray diffraction (XRD) analysis and optical microscopy.

2. EXPERIMENTAL

2.1 MATERIALS AND METHODS

Glucose oxidase (GOx, Aspergillus Niger extra pure) was purchased from Sisco Research Laboratories (SRL), India. Aniline (monomer) and ferric chloride (oxidant) of analytical grade were procured from Fisher Scientific, USA. Analyte glucose, glutaraldehyde solution (25%), urea, potassium dihydrogen orthophosphate, sodium hydroxide pellets (NaOH, 99%), sulphanilic acid were obtained from SD-fine Chemicals, India. All chemicals were used as received. The solutions used in the experiment were prepared using double distilled water.

Structural and morphological studies of PANI-S composite thin film deposited on fiber core were done by powder XRD using Rigaku diffractometer Miniplex II with nickel filtered CuKα radiations (λ=1.5406 Å) and optical microscopy (AxioCam, Germany). UV-vis spectroscopy was performed using StellarNet UV-vis spectrophotometer, USA in the wavelength range 190-800 nm. Sensing response of PANI-S composite cladding modified FOIGB was measured on CCD camera beam profiler (BC106-VIS, Thorlabs, USA).

2.2. FABRICATION OF FOIGB SENSING ELEMENT

Multimode plastic cladded silica optical fiber with 425/300µm core/cladding diameter optical fiber was used to direct the laser through sensing element to detector. Both the ends were cleaved and polished very well to enhance the coupling of light in the fiber. 2 cm portion at a middle of 1 m optical fiber was decladded to expose the core by mechanical means using stripper and surgical blade to fabricate the sensing element. The sensing element was prepared by dipping the cladding removed portion into a polymerization beaker solution containing aniline monomer with sulphanilic acid prepared in double distilled water. The drop wise addition of oxidant $FeCl_3$ at room temperature polymerizes aniline and deposit on core with sulphanilic acid as a dopant. The phosphate buffer solution of pH 7.4 was used to prepare GOx (2 mg/ml, 125 units/mg proteins) and glucose solutions. The GOx was immobilized on modified cladding through cross-linking via glutaraldehyde. After this, it was allowed to dry for 30 min and washed 2-3 times with phosphate buffer solution to leach out the loosely bound GOx molecules. The sensing element was rinsed in a phosphate buffer solution for 2-3 times to release the entire enzyme-GOx molecules that were not trapped inside or attached rigidly on the polymer matrix. He-Ne laser (632 nm, 2 mW) was coupled into the one end of the optical fiber and the change in power vs. time was recorded at the other end with the help of calibrated CCD camera beam profiler.

3. RESULTS AND DISCUSSION

3.1 UV-VIS ANALYSIS

Fig. 1 reveals the UV-vis spectrum of as-synthesized PANI-S film deposited on fiber optic core. It is very sensitive tool for the study of polymer and for the elucidation of the polymer chain. The PANI-S sample showed two absorption peaks at ~270 and ~500 nm. The peak at ~270 nm assigned to π-π* transitions and absorption peak at ~500 nm attributed to the transition between benzenoid-quinoid ring and conducting nature of PANI-S composite [11].

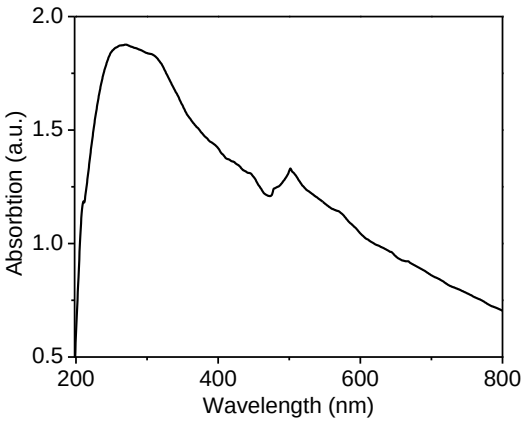

Fig. 1 UV-vis spectrum of PANI-S film.

3.2 XRD STUDY

The XRD pattern of PANI-S film recorded in the 2θ range 10 - 70° is shown in Fig. 2. The XRD pattern show few diffraction peaks related to scattering between adjacent surfaces of the benzene or adjacent chains of polymer that predict the semi-crystalline nature of prepared polymer PANI-S composite. The peaks related to the crystalline nature of oxidant are not appeared that indicates removal of the used oxidant and if present, it may be in dispersed form.

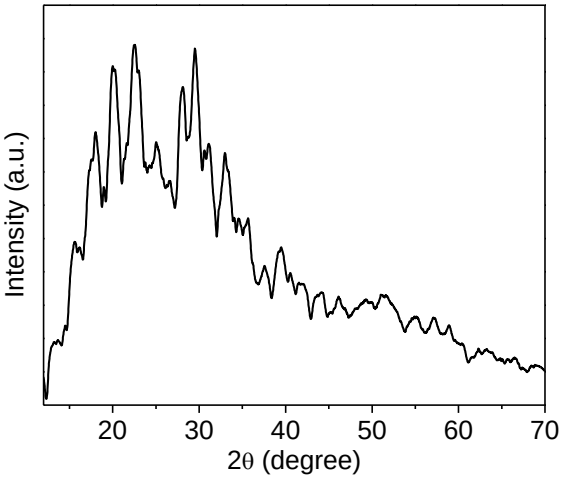

Fig. 2 XRD pattern of PANI-S composite

3.3 OPTICAL IMAGE

Fig. 3 represents the optical microscopic image of PANI-S thin layer deposited on sensing element of FOIGB. The thickness of the film was determined from it and found to be around 1.337 μm. It envisages the deposited thin layer on which GOx enzyme was immobilized to fabricate FOIGB.

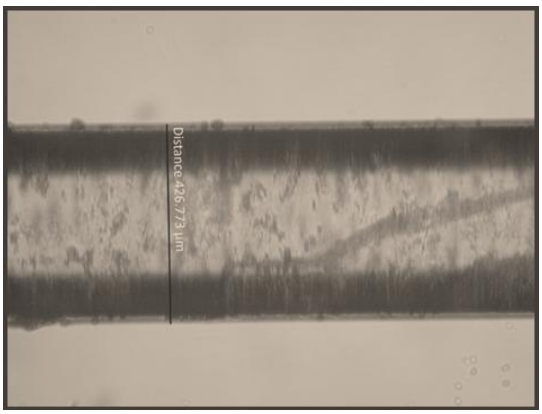

Fig. 3 Optical image of deposited PANI-S on core.

3.4 FOIGB RESPONSE STUDY

Fig. 4 demonstrates the performance of PANI-S composite cladding modified FOIGB. The sensor was evaluated at a room temperature by successive addition of phosphate buffer, 10 and 100 mM glucose solutions prepared in phosphate buffer of pH 7.4 and detecting optical power at the output. As shown in Fig. 4, with increase in the concentration of glucose in solution, well-defined increase in the power is observed. A trend of initial increases in the power and then becoming constant as time passes has been seen for all the solutions. It may be due to the reaction rate firstly increases and then saturates. The time required to achieve saturation power increases with concentration of glucose as it takes more time to attain equilibrium rate of reaction in high glucose concentration solution. When the glucose solution is introduced in the cell, the glucose molecules diffuses into the inner layer or get attached to the surface of the sensing film where GOx catalytically converts it into gluconolactaone and H_2O_2 in the presence of O_2 [3] that induce change in optical properties of sensing element and modify output signal accordingly. In the present study we have modified cladding with PANI-S composite and immobilized GOx on it via cross-linking and demonstrated sensing of glucose but not checked lowest detection limit. In addition to the checking lowest detection limit, other parameters like selectivity, response time and repeatability may be checked to decide applicability in real practice.

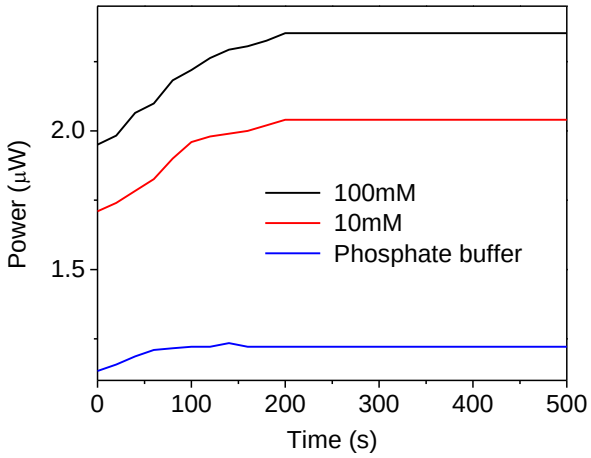

Fig. 4 Response curve of the FOIGB for phosphate buffer and for exposure of glucose solutions.

4. CONCLUSION

The PANI-S composite cladding modified FOIGB was developed by immobilizing GOx through cross-linking via glutaraldehyde. UV-vis spectroscopic, powder XRD and response studies have been presented. XRD study suggests PANI-S has semi-crystalline nature. The investigation also exhibits that polymer PANI-S composite may be applied as a potential cladding material for a variety of biosensor designs and used as a suitable candidate for enzyme immobilization. Present FOIGB showed ability and fast response toward glucose detection in solutions.

ACKNOWLEDGEMENT

This research work is financially supported by the Defense Research and Development Organization (DRDO), New Delhi, India (ERIP/ER/1003856/M/01/1293), which is gratefully acknowledged.

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