Optical Fiber Technology 40 (2018) 8–12

Contents lists available at ScienceDirect

Optical Fiber Technology

journal homepage: www.elsevier.com/locate/yofte

Regular Articles

Fabrication and evaluation of evanescent wave absorption based polyaniline-cladding modified fiber optic urea biosensor

S.N. Botewad, V.G. Pahurkar, G.G. Muley*

Department of Physics, Sant Gadge Baba Amravati University, Amravati, Maharashtra 444602, India

ARTICLE INFO

Keywords:
Optical fiber
Biosensor
Polyaniline
Cladding modification
Urease
Cross-linking

ABSTRACT

The fabrication and study of cladding modified intrinsic fiber optic urea biosensor has been reported in the present investigation. A simple cladding modification technique was used to construct the sensor by uncladding the small portion from optical fiber. Further bare core was decorated by supportive porous, chemically and optically sensitive matrix material polyaniline (PANI) as an active cladding for enzyme residency. Enzyme-urease (Urs) was cross-linked on the active cladding region via glutaraldehyde solution. Confirmation of the prepared PANI in proper form determined by ultraviolet-visible and Fourier transform infrared spectroscopic techniques. X-ray diffraction technique was employed for nature and compatibility examination of PANI. Sensor parameters such as sensitivity, selectivity, stability and lower detection limit have been analyzed by absorption variation study in evanescent wave field. The response of prepared sensor was studied towards urea in the wide concentration range 100 nM–100 mM and confirmed its lowest detection limit as 100 nM. The stability of sensor was found 28 days with little variation in response. The fabricated sensor has not shown any response towards interference species like glucose, ascorbic acid, L-alanine, L-arginine and their combination with urea solution and hence found selective for urea solution only.

1. Introduction

A simple, rapid, reliable, reproducible, sensitive and handheld device for urea estimation is in demand for medical as well as other various fields because widespread existence of urea everywhere [1,2]. Increase or decrease in the permissible level of urea concentration in human body causes various dangerous diseases such as congestive heart, urinary tract obstruction, gastrointestinal disorders, chronic or acute renal failure, burns, dehydration, starvation, shock, malnutrition, little dietary protein in the diet, liver diseases, hepatic failure, nephritic syndrome and cachexia [3,4]. In 1969 Guilbault et al. [5] were introduced a potentiometric enzyme electrode biosensor first time for urea determination. Nevertheless, environmental monitoring, health diagnosis, agriculture, food science, fishery industry, milk industry are waiting for a precise, fast and affordable instrument for urea detection [6–9]. For the fabrication of a biosensor, the deposition of enzyme on the electrode surface can improve the sensitivity, stability, response time and selectivity of biosensor. It can be achieved by selecting a specific immobilization technique [10]. Urea biosensors utilize enzyme-Urease (Urs) as the recognition element and reported as a highly efficient catalyst for the hydrolysis of urea, with a rate approximately 10^{14} times the rate of the non-catalyzed reaction [11]. Various useful aspects related to the urea biosensors have been cumulatively reviewed by Dhawan et al. [12] and Singh et al. [13]. Amperometric [14–18], potentiometric [19–21], conductometric [22–24], colorimetric [25,26] and optical [27–29] biosensors are useful for the urea determination.

The optical fiber based sensors are found more prominent, effective and convenient for chemical and biosensing purpose. Small and compact size, highly sensitive, reliable, fast response toward analyte, ability to be multiplexed, real time and parallel detection, remote sensing capability, immunity to electromagnetic interference, non-conducting and intrinsically safe for patients, etc., features make optical fiber sensor as an excellent candidate for biosensing [30,31]. Between two main extrinsic and intrinsic fiber optic types of sensors, cladding modified intrinsic fiber optic sensors are more advantageous because of their large dynamic range, high sensitivity, superior integration into other structures as well as convenient to prepare [32–35]. For the cladding modification, conducting polymers (CPs) such as polyaniline (PANI), polypyrrole, polythiophene and polyacetylene are being widely used because of their ability to provide stable and porous matrix for the immobilization of biomolecules. Moreover, catalytic reaction of enzyme-biomolecules on the sensing surface causes a change in redox and protonation state of CPs which directly useful for sensing response. This catalytic reaction may cause change in the microenvironment,

* Corresponding author.
E-mail address: gajananmuley@sgbau.ac.in (G.G. Muley).

http://dx.doi.org/10.1016/j.yofte.2017.11.002
Received 16 November 2016; Received in revised form 19 March 2017; Accepted 5 November 2017

surrounding the sensing element and triggers the sensing mechanism [36,37]. PANI has interesting electrochemical, electronic, optical, electro-optical as well as thermal and environmental properties for biosensor applications. It acts as an effective mediator for electron transfer in chemical and biochemical reactions. It makes a favorable porous suitable matrix for immobilization of biomolecules and easy to synthesize [37].

Present paper reports the fabrication of cladding modified fiber optic intrinsic urea biosensor (FOIUB) and its sensing response study. The cladding modification was achieved by chemically sensitive PANI matrix. Further enzyme-Urs was immobilized onto the PANI supporting matrix via cross-linking technique. Prepared biosensor offers relatively long self life, broad detection range, high sensitivity and specific selectivity towards urea. In the present paper we are reporting considerably lowest detection of urea as 100 nM.

2. Experimental

2.1. Materials and methods

For the PANI synthesis, aniline (monomer) and ferric chloride (oxidant) were purchased from Fisher Scientific, USA. The enzyme-Urs from jack beans, with activity 380 unit's mg^{-1}, was procured from Sisco Research Laboratories (SRL), India. Analyte-urea, cross-linking agent glutaraldehyde solution (25%), glucose, ascorbic acid, L-arginine and L-alanine were purchased from sdFine chemicals, India. For the preparation of buffer solution, potassium dihydrogen orthophosphate and sodium hydroxide were also purchased from sdFine chemicals, India. All the synthesis processes were carried out in freshly prepared double distilled water and in 0.1 M phosphate buffer solution (pH 7.4). All supplementary chemicals were of analytical grades and used as received without any further purification. The stock solutions of Urs in proportion 1 mg/3ml and urea were prepared in phosphate buffer (pH 7.4) and kept at temperature 4 °C for 24 h before use. The different concentrations of urea solution were freshly prepared at each time in the phosphate buffer of pH 7.4.

2.2. Characterizations

Deposited PANI film was characterized by powder X-ray diffractometer (XRD) (Mini Flex II, Rigaku, Japan) with CuKα radiations of wavelength 1.5406 Å to identify its morphology. The functional groups were confirmed by Fourier transform infrared spectroscopy (FT-IR) using α-ATR-IR-spectrophotometer (Brucker, Japan). Ultraviolet-visible (UV-vis) portable spectrophotometer BLACK-Comet-SR (Stellar Net, USA) in the spectral range 200–1100 nm was used for recording UV-vis spectrum of synthesized film as well as for the examination of sensing response of prepared sensor.

2.3. Cladding modification

Multimode optical fiber (40 cm long) of core/cladding dimensions 530/500 μm (1030 μm) of plastic cladded silica core was used for sensor fabrication. The SMA905 connectors were connected to both the ends of optical fiber using adhesive. Afterward both ends were furnished as well as polished using the fine polish papers of 1200 and 0.3 μm roughness respectively. Furnishing and polishing helps for enhancing the light gathering capacity of optical fiber [30]. Further, 2 cm cladding portion was removed from optical fiber with the help of stripper and surgical blade. Bare core surface was gently cleaned with hydrofluoric acid (HF) and distilled water. The active cladding was achieved by smoothly depositing uniform layer of PANI and kept it to settle. Synthesis of PANI was carried out by simple oxidative polymerization method using 0.2 M aniline monomer solution and 0.05 M $FeCl_3$ oxidant solution in 30 min reaction time. After dried the modified portion, it cleans with double distilled water to prepare the hydrophobic base

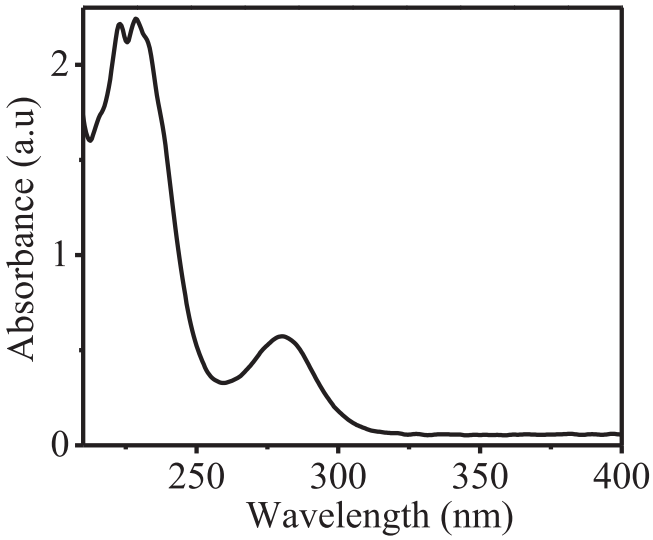

Fig. 1. UV-vis spectrum of PANI matrix.

for enzyme-Urs immobilization. Then using 1% glutaraldehyde solution as a homo-bi-functional cross-linking agent enzyme-Urs were immobilized on PANI modified sensing element. Sensing element was dried for 30 min at room temperature in clean environment and washed 2–3 times with phosphate buffer solution before it was used for sensing experiment. For unwanted effect of external light on the recorded sensing response, the experiments were carried out in dark room. The experimental arrangement adopted is reported in our previous article [38].

3. Results and discussion

3.1. UV-vis study

Fig. 1 shows the UV-vis spectrum of PANI film used to deposit as an active cladding of sensing element. The spectrum elaborates the different oxidation states of PANI. Three oxidation states occurs in PANI viz. reduced form leucoemeraldine, fully oxidized pernigraniline state and half oxidized emeraldine state. For the three different oxidation states in PANI, the nitrogen atom is responsible [39]. In present investigation PANI shows two characteristic peaks at ~227 and 280 nm. The absorption peaks below 300 nm correspond to π–π* transitions in the benzenoid ring distorted by the presence of amine groups which is characteristic of the leucoemeraldine form and pernigraniline form of PANI (π–π* transition in the quinoid ring) [40]. These two peaks ensure the synthesized PANI matrix is in proper form.

3.2. FT-IR study

Fig. 2 pictures the FT-IR spectrum of PANI along with its functional group frequencies and shows all the main characteristic peaks of PANI. The absorption peak at 713 cm^{-1} corresponds to the mono substitution of benzene ring and 1095 cm^{-1} stands for the C—H plane bending vibration [41]. The absorption peaks at 1237 and 1338 cm^{-1} may confirms the C—N stretching of primary aromatic amines. The strong absorption peak at 1720 cm^{-1} can be assigned to C═NH stretching. The peak at 1961 cm^{-1} may be attributed to the C—N bending and peak at 2103 cm^{-1} is of aromatic isonitrile N≡C stretching. The absorption peak at 2363 cm^{-1} may be due to aliphatic nitrile C≡N stretching. The absorption peaks at 2936 and 3067 cm^{-1} may corresponds to N—H stretching with hydrogen bonded amino groups and free O—H stretching vibration respectively. The absorption peak at 3429 cm^{-1} may be assigned to asymmetrical and symmetrical stretching vibrations of NH_2 group [42]. Thus, the FT-IR spectrum shows all expected