



ELECTROCHEMICALLY SYNTHESIZED PANI/ GLUCOSE OXIDASE THIN FILMS FOR ELECTROCHEMICAL GLUCOSE DETECTION APPLICATION

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ABSTRACT:

In present context we have synthesized PANI/ Glucose oxidase thin films were prepared by template free, simple one step potentiostatic method. Prepared films were characterized by the structural study by FT-IR, morphological study by FE-SEM and electrochemical analysis by cyclic voltammetry, I-t plot. And enzymatic activity by finding Michel-Menten Constant, LOD. Present work shows 257.37 $\mu\text{A cm}^{-2} \text{mM}^{-1}$ current sensitivity which is much enhanced than reported one

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1. INTRODUCTION

Due to excellent properties like air stability, biocompatibility and ease of preparation of polyaniline (PANI) it is mostly used in many application as potential candidates [1]. Amongst various application biosensing capturing more attention specially as PANI glucose biosensors for detection of glucose because ease of generation and offers uniform nature of thin films [2]. In addition to this PANI possesses two oxidation and two reduction peaks in particular potential range which helps in direct electrochemical between the enzymes (Glucose oxidase) from PANI electrode surface to electrolyte. PANI biosensors itself directly perform electrocatalytic activities in analyte solution without any diffusion mediators [3]. Moreover, PANI/ Glucose oxidase biosensors eliminates the shortcomings occurred in the second generation of glucose biosensor like enzyme fouling during electrochemical performance and barrier in diffusion mediator which present at the interface of the PANI/Glucose oxidase thin film. This process provides the longer stability of polymer enzyme electrode [4]. Several research groups were reported a variety of form of PANI morphologies regarding to their use for application purpose like nano tubes, nano wires, nano fibers, nano grains, rods like morphology based on the synthesis method and deposition parameters. Due to diversities in surface structure/ morphologies PANI permit fastest diffusion of enzyme molecules in the electrolyte solution [5]. However, the combined structural and electrocatalytic properties play a vital

role bio sensing performance by minimization of charge transfer resistance [6, 7].

In present context we have synthesized PANI/ Glucose oxidase thin films for different deposition potentials viz. 1.0, 1.1 and 1.2 V vs. Ag/AgCl for electrochemical biosensor application. The PANI/Glucose oxidase thin films were prepared by template free, simple one step potentiostatic method and the immobilization of Glucose oxidase enzymes electrochemical polymerization which entraps Glucose oxidase enzymes into the PANI matrix directly. Later all prepared PANI/Glucose oxidase thin films were employed for the different physicochemical techniques. FE-SEM study reveals the morphological transformation from interconnected fibrous network structure to randomly dispersed nano rods. FT-IR study provides the chemical structure confirmation and rest the electrocatalytic performance comprises CV, EIS study, amperometric response enzyme kinetic activity parameter like Michaelis constant etc.

3. RESULTS AND DISCUSSION

3.1 EXPERIMENTAL SETUP:

The schematic diagram for experimental setup of electro deposition cell is shown in Fig. 4.1. It consists of three electrode system, i.e. any conducting substrates (ITO) as anode, counter electrode as cathode, reference electrode, back elite holder and deposition cell etc. The deposition of

PANI films were performed using aniline monomer and sulphuric acid of the quantity mentioned in synthesis section.

PANI/Glucose oxidase films were synthesized at different applied potentials 1.0, 1.1 and 1.2 V for 1500 s keeping the aniline monomer concentration and H₂SO₄ electrolyte concentration constant. The PANI deposited at above potentials was taken as working electrodes throughout the experiments. The Pt wire and Ag/AgCl electrode were used as counter and reference electrodes. The enzyme immobilization is as important task while constructing PANI/Glucose oxidase electrodes for electrochemical biosensor. In present study the Glucose oxidase enzyme immobilization was performed by enzyme entrapment method during electropolymerization of aniline. During this process the negatively charged Glucose oxidase molecules were entrapped in PANI matrix. The preparative parameters for synthesis of PANI/Glucose oxidase thin films were given below in table,

2. EXPERIMENTAL DETAILS:

2.1 SOLUTION PREPARATION:

Glucose oxidase solution, aniline monomer, PBS buffer solution, H₂SO₄ (99 %) were purchased from Sigma - Aldrich.

2.2 SYNTHESIS:

To synthesize the PANI/ Glucose oxidase thin films by electrode position mode three electrode system was used which comprises ITO (1 x 1 cm²) which serve as working electrode, Pt wire acts as auxiliary electrode and silver/ silver chloride as reference electrode was employed to perform the experiment. PANI/Glucose oxidase thin films were papered by one step electrode position method using 0.2 M equimolar 45 ml deposition solution of aniline monomer solution and sulphuric acid for deposition potentials 1.0, 1.1 and 1.2 V vs. Ag/AgCl. After that 5 ml of 2 mg/ ml was added to the deposition solution. All the films were synthesized for the optimised time parameter of 500 s. In addition to this the enzyme immobilization was performed during electro polymerization i.e. by enzyme entrapment method. This method of enzyme immobilization is advantageous than the rest mentioned method

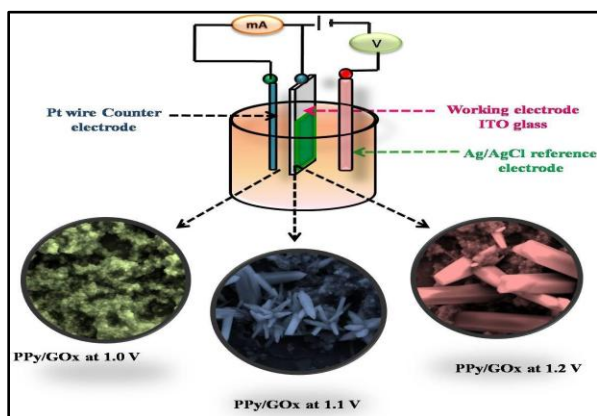


FIG.1 EXPERIMENTAL SETUP FOR

ELECTRODEPOSITION OF PANI / GLUCOSE OXIDASE THIN FILMS

3. RESULTS AND DISCUSSION:

3.1 FT- IR STUDY:

The FT - IR spectra of PANI/Glucose oxidase for the deposition potentials 1.0, 1.1 and 1.2 V was represented in the Fig. 2 (a, b and c) respectively. IR peaks noticed at 1561, 1484, 1295, 1240, 1077 and 792. 4 cm⁻¹ which are associated with chemical bonds C - C stretching, C - N stretching, N - H vibration mode, C - H plane bending vibration respectively [16-23]. The impact of deposition potentials on the peak intensity is clearly seen in the Fig 2 of the PANI/Glucose oxidase thin films. Amongst all the three recorded FT - IR spectra (2 a, b and c), spectrum 2 b shows relatively prominent and intense absorption peaks. This because of Glucose oxidase molecule got attach to N - H group of PANI structure. This is due to maximum entrapment of Glucose oxidase molecules within PANI matrix and successful immobilization of Glucose oxidase occurred.

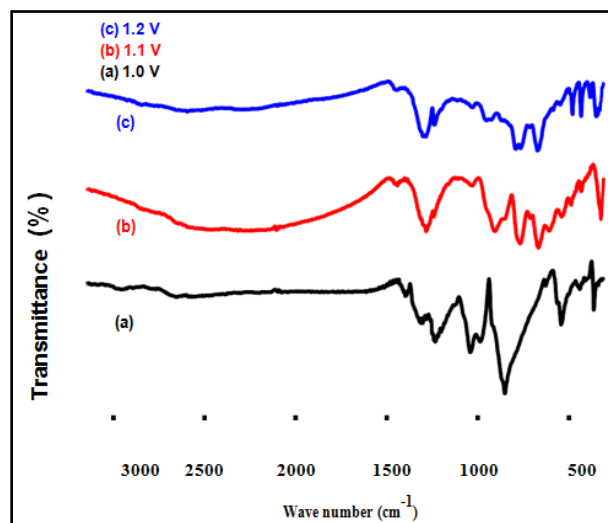


FIG. 2 FT-IR SPECTRA OF PANI/GLUCOSE OXIDASE THIN FILMS

FE - SEM pictures (Fig. 4.3 - a, a', b, b' and c, c') of prepared PANI/GOx thin film for different deposition potentials viz. 1.0, 1.1 and 1.2 V respectively for different magnifications. The surface characteristic of the deposited film showed in Fig. 4.3 (a, a') reveals interconnected fibrous structure with whitish GOx crystals engulfed within PANI matrix. The surface morphology of PANI/GOx traced for the film synthesized at 1.1 V [24] results in the dent corn like structure embedded in the fibrous interconnected PANI matrix of length about 150 nm and diameter 100 nm. This kind of morphology provides large area to interact with glucose during glucose sensing. The peaks depicted in FT -IR results also support the FE - SEM results showing that the impact of change in potential and due to presence of GOx in PANI matrix, prominent absorption peaks may lead increase in the length of rods and dent corn like formations.

Furthermore, upgrading the deposition potential as 1.2 V gave nano rods of length 1 micron with diameter of 200 nm which are also clearly seen embedded into PANI matrix. The variation occurred in the surface morphology due to increment in the deposition potential showed in Fig 4.3 (c, c'). As comparing the surface morphologies of PANI/GOx constructed for different potentials gave drastic transformation in appearance. About all the three (Fig. 4.3 - a, a', b, b' and c, c') morphology obtained for 1.1 V deposition potential showed more porous structure than the rest of surface morphologies recorded since this kind of structure provides more electrode surface and electrolyte interaction during bio sensing mechanism.

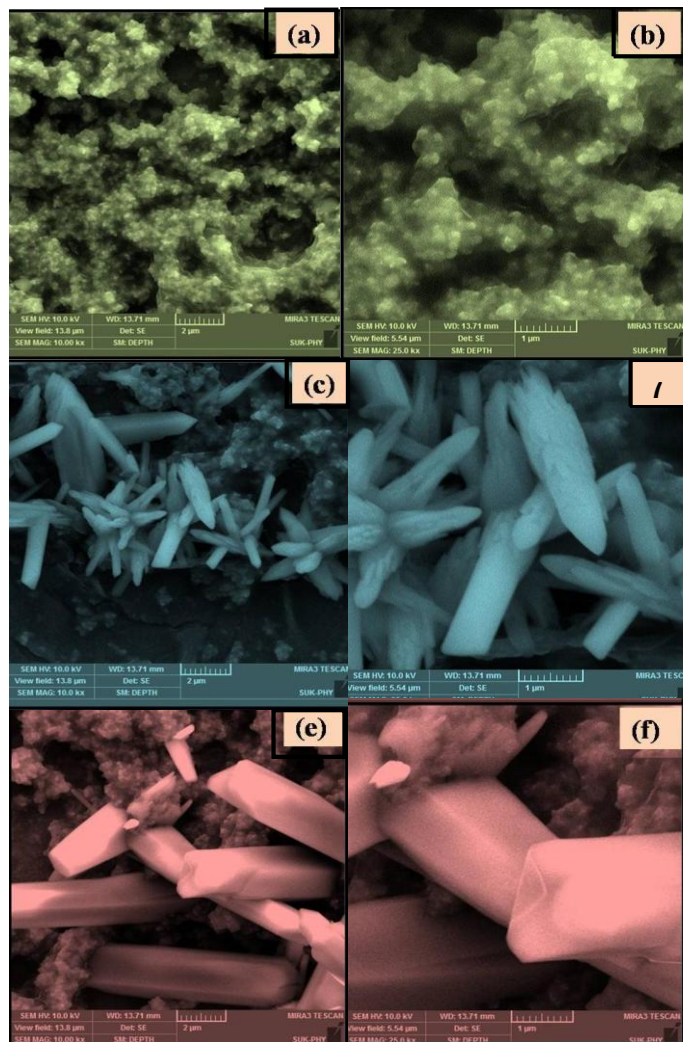


FIG. 3 FE-SEM MICROGRAPHS OF PANI/GLUCOSE OXIDASE THIN FILMS SYNTHESIZED AS 1.1 V (A, B) 1.2 V (C, D) AND 1.3 V (E, F)

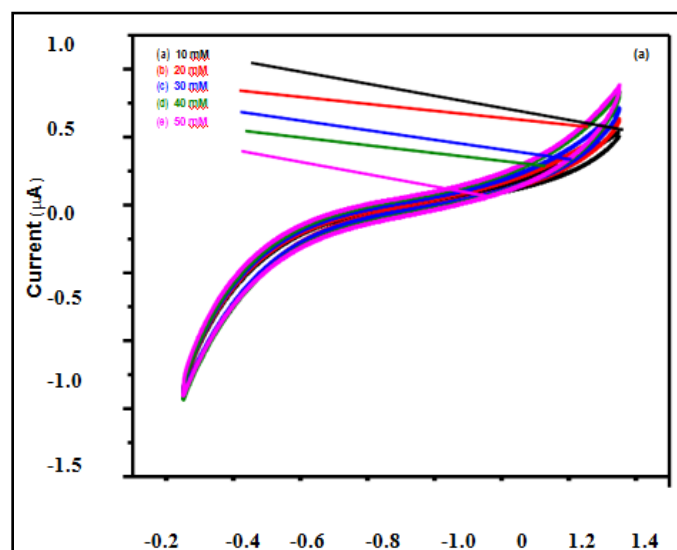


FIG. 4 PREPARED FILMS WERE TESTED IN BLANK SOLUTION OF 0.1 MM PBS SOLUTION.

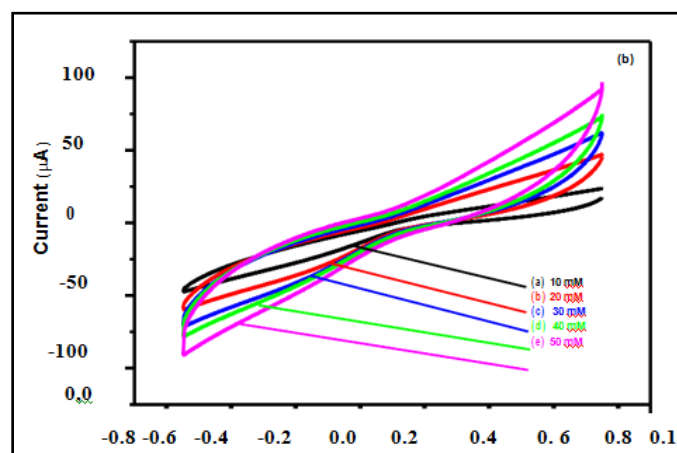


FIG. 5 CURRENT RESPONSE FOR VARIOUS GLUCOSE CONCENTRATION CURVES A) 10 MM, B) 20 MM, C) 30 MM, D) 40 MM AND E) 50MM

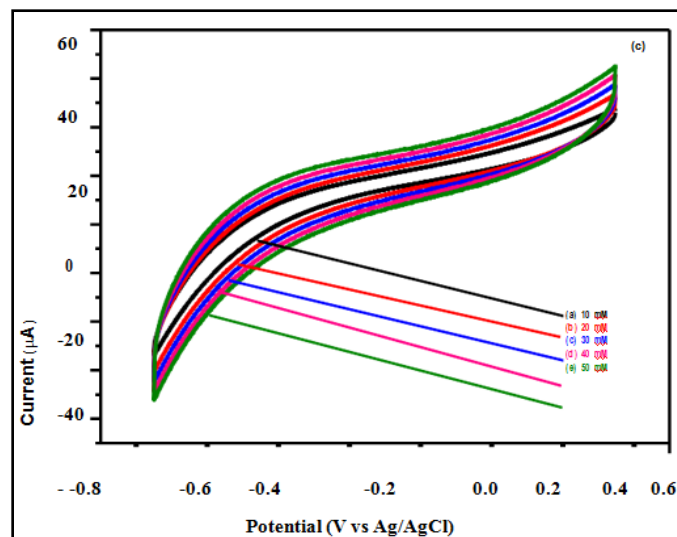


FIG. 6 CYCLIC VOLTAMMOGRAMS OF THE PANI/GLUCOSE OXIDASE THIN FILMS

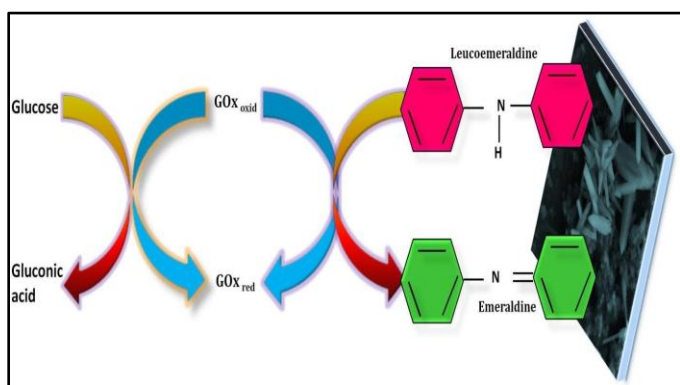


FIG. 7 BIOSENSING MECHANISM OF PANI/GLUCOSE OXIDASE THIN FILM

3.2 EIS STUDY:

Fig. 8 (a, b and c) shows the EIS spectra of deposited films. The hemispherical plot of PANI/Glucose oxidase corresponding to higher frequencies and straight-line comprises lower frequency for all the synthesized films. The bigger hemispherical curve was obtained for the film deposited at 1.2 V Fig. 8(c) which was resulted in highest resistance of electron transportation. By making comparison between EIS curves b and c, the curve noticed at 8 b shows relatively decreased in hemisphere diameter of PANI/Glucose oxidase thin film. Furthermore, the noticeable decay in the diameter had seen for the PANI/Glucose oxidase deposited 1.1 (Fig. a). The linear shape of EIS diagram results the low electron transfer resistance. The diameter of semicircle associated with PANI/Glucose oxidase deposited at potential 1.1 V was bigger than that of the PANI/Glucose oxidase deposited at potentials 1.0 and 1.2 V shown in Fig. 8(a and c). It suggests that the PANI/Glucose oxidase films at 1.1 V the immobilization and the molecules entrapped in PANI matrix is more than the rest potentials. The reason is that the randomly oriented dent corn like of PANI was obstructed the conductivity of the modified electrode

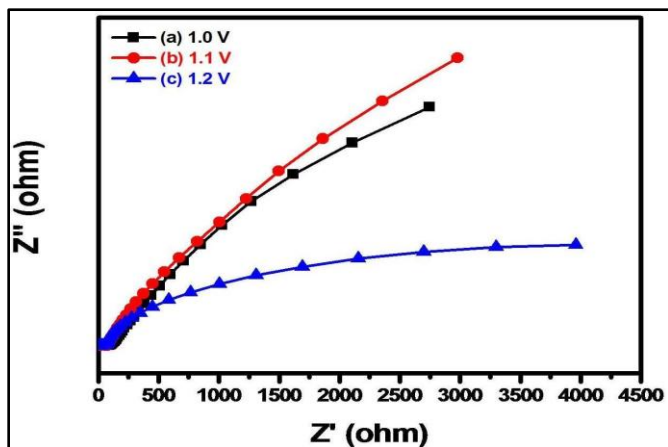


FIG. 8 EIS SPECTRA OF PANI/GLUCOSE OXIDASE THIN FILMS

3.2.1 AMPEROMETRIC RESPONSE:

The typical amperogram of optimized PANI/Glucose oxidase thin film deposited at 1.1 V potential is shown

below Fig. 9 It is clearly seen from graph that successive addition of 10 mM of glucose gives rise to increment in current with time. After 60 mM of glucose concentration current response stabilizes with time. The response time noticed for PANI/Glucose oxidase electrode is 10s

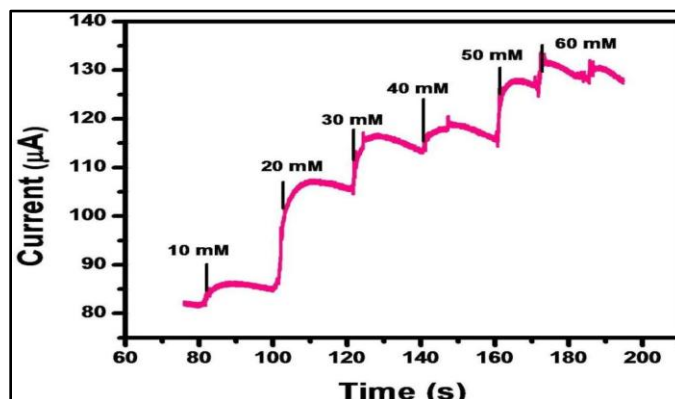


FIG. 9 AMPEROGRAM FOR OPTIMISED PANI/GLUCOSE OXIDASE FOR 10 MM GLUCOSE

4.3.7. MICHAELIS -MENTEN (KM) CONSTANT:

The Km value for PANI/ Glucose oxidase was recorded from the graph shown in Fig. 10 Km is calculated to be 22.78 µM for optimised PANI/Glucose oxidase film at 1.1 V which is analogous to that of reported native Glucose oxidase enzyme value [26]. The nondenaturation temperament of immobilization of the Glucose oxidase was confirmed. Lower the Km poor the kinetic performance of Glucose oxidase molecules to glucose electrolyte interaction.

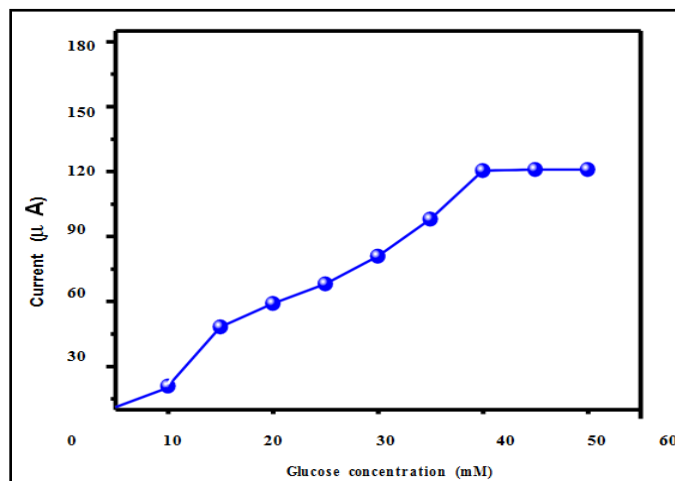


FIG. 10 CALIBRATION CURVES OF PANI/GLUCOSE OXIDASE

Figure 10 shows a typical calibration graph of PANI/Glucose oxidase thin films as biosensor at deposited at 1.1 electrode. This plot is used to determine the glucose concentration. The PANI/Glucose oxidase electrodes at 1.0 V displayed the excellent performance. The linearity of graph is viewed in the range 10 to 70 mM of glucose concentration with $R^2 = 0.9969$. The lowest limit of detection was recorded 1.0 µM of glucose for present study with S/N= 3. Reciprocal of the linear current versus

glucose concentration is Line weaver plot (Fig. 10) of synthesized electrode. Sensitivity of the optimised electrode is found to be $257.37 \mu\text{A cm}^{-2} \text{mM}^{-1}$ [25].

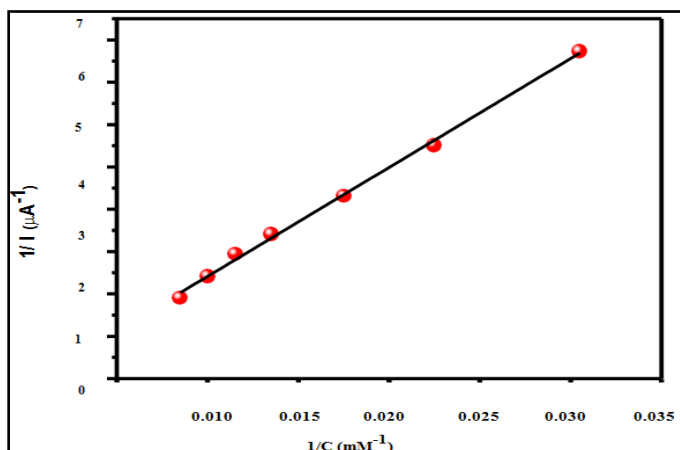


FIG. 11 LINEWEAVER-BURK PLOT OF I-1 VS. [GLUCOSE]-1 OF PANI/GLUCOSE OXIDASE THIN FILM

4.3.8 STABILITY AND LIFETIME:

The stability of the PANI/Glucose oxidase was examined via the amperometric response of glucose for optimized PANI/Glucose oxidase electrode shown in Fig. 11. The optimized PANI/Glucose oxidase electrode was tested for 10 mM glucose independently for the time interval of 15 days. It specifies that good stability with every test along with efficient Glucose oxidase in PANI matrix. After the each stability check the PANI/Glucose oxidase electrode stored at 4°C . It prevents the enzyme fouling. The biosensor maintained 25 and 65 % of its glucose sensitivity after 7 and 15 days, respectively. The good stability of the biosensor, results in the intrinsic stability of immobilized Glucose oxidase in the PANI polymer matrix. This stability obtained for present work shows better result than the LDHs/GOD biosensor [21-23]

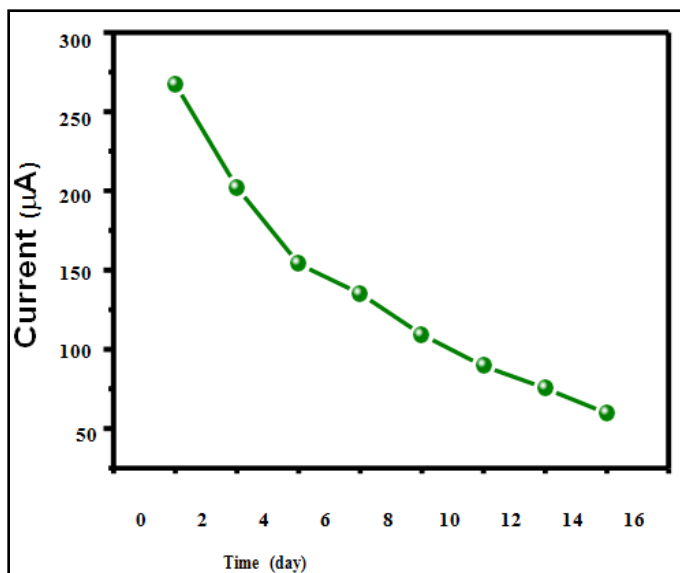


FIG. 12 THE STABILITY CURVE OF PANI/GLUCOSE OXIDASE THIN FILM

4.4 CONCLUSIONS:

In summary, the investigations made in this work show the different applied potential offered during construction of the PANI/Glucose oxidase thin films. Moreover, the usage of enzyme entrapment immobilization technique; the problem of leaching of Glucose oxidase during experiments were avoided. The PANI/Glucose oxidase electrode is optimised at 1.1 V by considering the current sensitivity parameter. The optimised PANI/Glucose oxidase electrode is used to study the various glucose concentrations in the range of 10-50 mM in 0.1 M PBS (pH = 7) and sensitivity $257.37 \mu\text{A.mM}^{-1}.\text{cm}^{-2}$ with LOD = $1 \mu\text{M}$ is evaluated. The well defined specificity and stability towards glucose exhibited by the PANI/Glucose oxidase electrode is due to the biocompatibility and the adhesive ability of the PANI/Glucose oxidase film were confirmed by its characterization by means of FT - IR, FE - SEM and electrocatalytic studies cyclic voltammetry, electrochemical impedance and amperometric response, etc.

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