

Cyclic Voltametry Study of PANI/ZnOUrease Based Biosensor with Stainless Steel Electrode as Transducer

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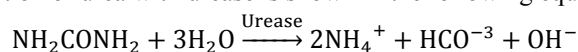
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Abstract: The electrochemical characteristic Cyclic Voltametry (CV) was studied for Electrodeposited Polyaniline (PANI)/ZnO nanocomposite onto a stainless steel transducer. The Cyclic Voltametry was studied during electrodeposition of PANI/ZnO on the stainless steel electrode. Good cyclic voltammogram for 15 wt% composition was observed as compared to others. The PANI/ZnO film also offers a more symmetrical voltammogram, with oxidation starting at a lower potential (0.05 to 0.08 V) compared to pure PANI, indicating that the presence of the ZnO promotes the electron-transfer of the oxidation process. Cyclic Voltametry of PANI/ZnO/Urease electrode shows the oxidation potential peaks occurs in CV of ZnO-PANI 15% film depicts the oxidized potential at around 0.2V, which is at higher potential as compared to other lower weight %, indicating larger surface area and larger potential window as compared to others. The magnitude of peak current gets increased with increasing concentration of ZnO, which ensure quick response time of the sensor. The CV of PANI/ZnO/Urease in potential window of -0.1 to 0.1V shows resistive effect of PANI. Also, the cyclic voltammogram of PANI/ZnO/Urease 15% shows more ohmic behavior as compared to other compositions and PANI.

Keywords: Polyaniline, Zinc oxide, Urease, Cyclic Voltametry, Stainless steel, biosensor

I. INTRODUCTION

The detection of heavy metal ions in natural water, soil and air has become very important because of their accumulation and storage in living organisms may cause serious disorder in the metabolic activities. Recent research has shown importance of biosensors in the detection of these hazardous heavy metal ions. The transducer is a key component in the construction of biosensor. A certain number studies have been reported for the use of interdigitated thin film electrodes [1], Titanium, Chromium, Aluminium, Platinum, mild steel [2,3] etc as a transducer element. But, these electrodes are undesirable for operation with biological liquids since these electrodes have low sensitivity to changes in the ion strength of solution and reach conductivity saturation in a short time. The stainless steel electrodes are however less costly and can easily be available. The polyaniline can easily deposited on stainless steel by electropolymerization [4]. The Electropolymerized layer on stainless steel electrode immobilized with the enzyme gave water-insoluble, transparent film, with a high enzyme activity [5]. The advantage of nanostructure ZnO [6] used modified transducer surface as compared to other metal oxides such as CeO₂, SnO₂, TiO₂, FeO₂ and ZrO₂ is their unique ability to promote faster electron transfer between electrode and active site of desired enzyme. The ZnO nanostructures are an important widely used due to their unique properties including high specific surface area, high catalytic efficiency, strong adsorption ability, high isoelectric point (IEP 9.5), wide band gap (3.37 eV), biocompatibility and high electron communication features [7]. Also, less toxicity, high chemical stability and high electron transfer capability make ZnO as a favorable surface for immobilization of enzymes such as Urease. Since biosensors are designed by using biological elements such as enzymes, they can be inhibited by heavy metal ions [8]. Generally, urease was used as the enzyme inhibitor by heavy-metal ions [9, 10, 11] because of its low cost easy availability. The enzymatic reaction of urea with urease is shown in the following equation



The reported sequence of metals ions relative to their toxicity towards urease is:

$\text{Hg}^{2+} > \text{Ag}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Pb}^{2+} > \text{Mn}^{2+}$ [12, 13, 14, 15].

Cyclic voltammetry (CV) is the most versatile electro analytical technique for the mechanistic study of redox behavior, number of electrons involved in the redox reaction, electrochemical studies, degradation studies, diffusion coefficient estimation and study of reversibility of redox couples in conducting polymer systems [16, 17].

The present study aims to make evident the electrochemical behavior of the aniline with stainless steel electrode surface in acid medium by cyclic voltammetry within a potential range of -0.2 to $+0.8\text{V/S.C.E.}$ and a scanning rate of 50 mV s^{-1} . Also, Cyclic voltammetry properties for determining oxidization potential, response time and effect of weight % of ZnO on the performance of prepared biosensor.

II. EXPERIMENTAL

2.1 Materials

All chemical used were of analytical grade, Aniline, ammonia solution (min 25%), zinc nitrate (96%), acetone, from Merk Ltd, Mumbai, Urease (Jack bean mill) from Lobachemie, were used as received without further purification. Doubly distilled water was used throughout this work.

2.2 Synthesis of Nano-sized ZnO

The sample of pure zinc oxide compound was prepared by co-precipitation method. The solution of 0.2M of zinc nitrate $[\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ was prepared in distilled water and to this solution ammonia solution was added drop wise till the pH adjusted to 8. The hydrated zinc hydroxide gel thus formed was thoroughly washed with distilled water and transferred to flask fitted with water condenser. The gel was continuously stirred for 6 hours and temperature was maintained around 85°C . then, the nanocrystalline ZnO powder was filtered and oven dried.

2.3 Electropolymerization of PANI/ZnO Nanocomposite

For electropolymerization of aniline, different weight percentage of nanostructure ZnO (5%, 10% and 15%) were added in 1 M H_2SO_4 containing of 0.4 M aniline. The electrodeposition was performed using Potentiostat model CH-600 D. Platinum wire was used as Counter electrode, Ag/AgCl₂ as reference electrode and Stainless Steel as working electrode. The layer of PANI/ZnO nanocomposites with different weight percentage were deposited on Stainless Steel surface by linear swiping the voltage in range 0.2 to 0.8 volt at the scan rate of 50 mVs^{-1} for 50 cycles. The electrodes are washed with distilled water and dried at room temp and used for further studies. For comparison, pure PANI was also polymerized in the same condition as mentioned above, but ZnO was not added in the polymerization electrolyte.

2.4 Enzyme Immobilization

The immobilization of Urease on PANI /ZnO matrix on Stainless steel electrode was done using Physical Adsorption method. The electrodes were dipped in a pH 4 acetic acid solution, washed with water and then left overnight at 5°C in contact with an urease solution containing 2 mg of the enzyme (urease) per ml of pH 5.6 phosphate buffer. The next day, the membrane was washed with a pH 7 phosphate buffer solution. The prepared PANI/ZnO/Urs electrodes will be always stored dry at 4°C [18].

III. RESULT AND DISCUSSION

3.1 Mechanism of PANI Film Formation by Electropolymerization

The voltammogram indicates oxidation proceeding on stainless steel surface in two stages. The anodic peak situated at more positive potentials ($E_{pa} = +0.3\text{ V/S.C.E.}$) is particularly intense in the anodic branch of the first spinning cycle and can be attributed to the initial monomer oxidation. The neutral aniline molecule is oxidized and deprotonated in the first stage, when a nitrenium cation $\text{C}_6\text{H}_5\text{NH}^+$ is formed. By the electrophilic attack of this cation on the other aniline molecule, a "head-tail" dimer (*p*-minodiphenylamine) is formed. The dimer formation is the rate determining step, which is accompanied by the loss of an electron (e^-) and of a proton for every aniline molecule increasing rate of polymer chain, of about 10^4 times higher than the initiation one [19].

The dimer is then oxidized in one step to the diiminequinoiden form, with participation of two electrons, due to its low oxidation potential in mcomparison with aniline. The redox couple situated within the range of less positive potentials has a progressive evolution (intensities of Voltammetric currents increase with increasing the number of scanning cycles) and might be attributed to the dimer and, subsequently, to the polymer forming on the stainless steel electrode surface [20]. In cyclic voltammetry studies, the “nucleation loop” commonly observed on the first scan is characteristic of conducting phase formation by a nucleation and growth mechanism.

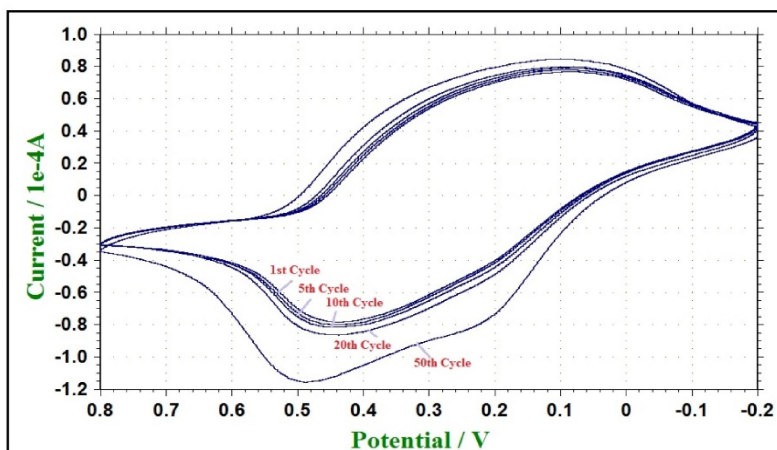


Figure 3.1: Growth of PANI on SS electrode.

From the **Fig. 3.1** it is observed that, the anodic–cathodic peaks of the polymer (5th cycle onward) are narrower than anodic peak of monomer (1st cycle). This indicate that the charge transfer in the case of the monomer is small than in the case of the polymer. This will lead to high conductivity of the polymer layer formed at electrode surface. In the 5th cycle one oxidation peak is observed at 0.51 V vs Ag/AgCl which was related to the oxidation of aniline [21].

3.2 Co-deposition of PANI/ZnO nano Composite by Cyclic Voltammetry

PANI/ZnO nano composite film was electrochemically synthesized by electrolyzing aniline, different weight percentage of nanostructure ZnO (5%, 10% and 15%) in 1 M H₂SO₄ containing of 0.4 M aniline on the steel electrode by sweeping the potential on the working electrode, from –0.2 to +0.8 V versus Ag/AgCl for 50 cycles with a scan rate of 50mV/s. Repeated cycling of the potential resulted in continuous deposition of PANI/ZnO film onto the electrode surface. Good cyclic voltammogram for 15 wt% composition was observed as compared to others. Therefore, only 15 wt% composition is further studied and reported.

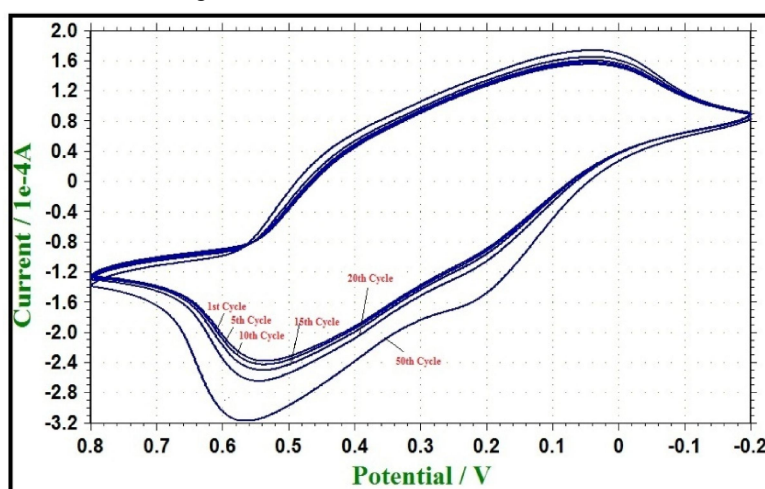


Figure 3.2: Growth of PANI/ZnO 15% on SS electrode

Fig. 3.2 shows voltammograms of synthesis of PANI/ZnO 15% composition. It can be seen from Figure that a polymer growth, with increasing current is observed in the presence of nanostructure ZnO. Thus, the ZnO acts as the counter ion incorporated within the polymer film to balance the cationic charge of the oxidized polymer [22]. The PANI/ZnO film also offers a more symmetrical voltammogram, with oxidation starting at a lower potential (0.05 to 0.08 V) compared to pure PANI. (Figure. 3.3, where oxidation occurs at 0.51V), indicating that the presence of the ZnO promotes the electron-transfer of the oxidation process [23].

3.3 Cyclic Voltammetry of PANI/ZnO/Urease electrode

Fig. 3.3 (a) shows the cyclic voltammograms of PANI/ZnO/ Urease electrode with 5%, 10% and 15% compositions in phosphate buffer solution. Cyclic voltammograms were obtained by sweeping the potential on the working electrode, from -0.2V and 0.8 V versus Ag/AgCl at a scan rate 100 mV/s. The oxidation potential peaks occurs in CV of ZnO-PANI 15% film depicts the oxidized potential at around 0.2V, which is at higher potential as compared to other lower weight %, indicating larger surface area and larger potential window as compared to others. The magnitude of peak current gets increased with increasing concentration of ZnO, which ensure quick response time of the sensor. The CV of PANI/ZnO/Urease in potential window of -0.1 to 0.1V shows resistive effect of PANI. Also, the cyclic voltammogram of PANI/ZnO/Urease 15% shows more ohmic behavior as compared to other compositions and PANI [24].

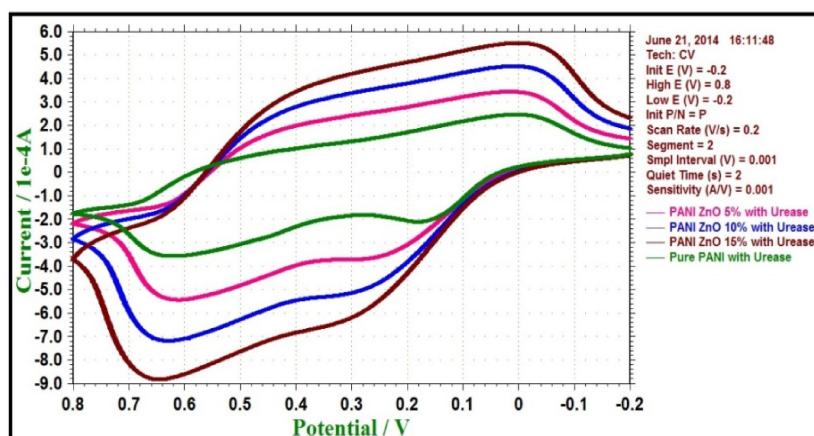


Figure 3.3 (a) CV of PANI/ZnO/ Urease electrode

The effect of Urease is shown in **Fig. 3.3 (b)**. From the cyclic voltammograms it is observed that the magnitude of peak current get increased with incorporation of Urease on PANI/ZnO 15%. This effect attribute to the quick response of the constructed biosensor [25].

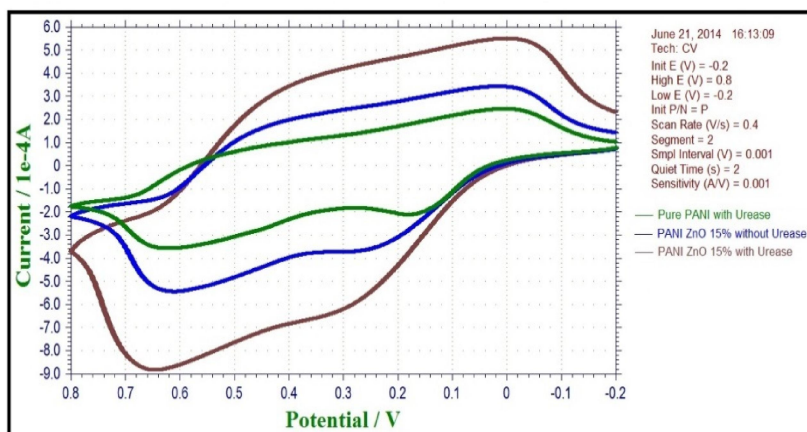


Figure 3.3 (b) Effect of Urease on the PANI/ZnO 15% electrode surface

IV. CONCLUSION

The CV of PANI/ZnO/Urease in potential window of -0.1 to 0.1V shows resistive effect of PANI. On the other hand the cyclic voltammogram of PANI/ZnO/Urease 15% shows more ohmic behavior as compared to other compositions and PANI. The immobilized enzyme by physical adsorption method is well entrapped in to the PANI/ZnO matrix and it will not show degradation of the Urease from the electrode surface. The PANI/ZnO(15%) has found a lower electrochemical charge transfer resistance which results fast charge transfer in the electrolyte compared to that of the pure PANI. The SEM image reveals that in PANI/ZnO composite shows ZnO had a strong effect on PANI morphology and a transformation in morphology from typical agglomerated form of PANI to particles with high porosity. PANI/ZnO/Urease shows the entrapment of urease globular structure in to the prepared PANI/ZnO matrix. The results of Cyclic voltametry show that this biosensor exhibits an excellent response for urea at working potential of -0.3 V to 0.6 V.

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