Study of Polyaniline/ZnO Nanocomposite Electrodeposited On Stainless Steel Electrode For Biosensor Application

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***Abstract*— Electrodeposition of chemically synthesized Polyaniline (PANI)/ZnO nanocomposite onto a stainless steel transducer was studied for amperometric biosensor application. The electrodeposited polymer matrix of PANI and nanostructure ZnO in different weight percent (5%, 10%, 15%) in acidic medium was used for immobilization of Urease by physical adsorption method. PANI/ZnO nanocomposites were characterized by Electrochemical Impedance Spectroscopy (EIS), SEM, FTIR and XRD. The spectra obtained from these characterizations indicate the strong interaction of nanostructure ZnO with PANI matrix. The prepared PANI/ZnO/Urs biosensor was studied by plotting urea calibration curve. The calibration curve shows that the amperometric current increases linearly with increase in Urea concentration. This will provide the better sensitivity and stability of the prepared biosensor. The storage stability curve shows the response of the biosensor when stored in air was very poor even on next day of preparation of biosensor. This may be due to high reactivity of Urease with atmospheric air. So this study recommends that the PANI/ZnO/Urs biosensor should be stored in buffer solution at 4^0C.**

***Keywords*— Polyaniline, Zinc oxide, Nanocomposite films, Urease, Hg(II) ions in water, Stainless steel**

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. Out of all, effect of mercury on environment is especially very serious due its toxicity and wide range of industrial use. Heavy metals are typically analyzed by traditional methods, including inductively coupled plasma atomic electron spectrometry (ICP/AES), inductively coupled plasma mass spectrometry (ICP/MS), atomic absorption spectroscopy (AAS) or wet chemical methods [1]. These conventional methods have several disadvantages as they are laborious, time consuming and need expensive instruments and sample pre-treatment. Biosensors present an efficient alternative technique which overcomes many of these disadvantages, such as minimum sample pre-treatment and less technical training [2]. 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 [3], Titanium, chromium, Aluminium, Platinum, mild steel [4]; [5] etc as a transducer element. But, Titanium, chromium and Aluminium 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 interdigitated thin film electrodes and Platinum are costly and not available easily. The stainless steel electrodes are however less costly and can easily be available. The Polyaniline can easily deposited on stainless steel by electropolymerization [6]. The Electropolymerized layer on stainless steel electrode immobilized with the enzyme gave water-insoluble, transparent film, with a high enzyme activity. Various enzymes remain sufficiently entrapped in the polymer matrix [7].

Due to the unique properties of nanostructures in the biosensing area, nanosensors offer some significant advantages owing to their small size and high surface area to volume ratios allowing larger signals, better catalysis and the more rapid movement of analytes through sensors. The advantage of nanostructure ZnO [8] 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 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 [9]. Also, less toxicity, high chemical stability and high electron transfer capability make ZnO is favorable 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 [10]. Generally, urease was used as the enzyme inhibitor by heavy-metal ions [11]; [12]; [13] because of its low cost easy availability. The enzymatic reaction of urea with urease is shown in the following equation.

$$NH_2CONH_2 + 3H_2O \xrightarrow{Urease} 2NH_4^+ + HCO^{-3} + OH^-$$

II. Material characteristics

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 Loba chemie, were used as received without further purification. Doubly distilled water was used throughout this work.

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 [Zn (NO3)2 6H2O] 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 85o C. then, the nanocrystalline ZnO powder was filtered and oven dried.

Electropolymerization of PANI/ZnO nanocomposite: For electropolymerization of aniline, different weight percentage of nanostructure ZnO (5%, 10% and 15%) were added in 1 M H2SO4 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. After 50 cycles the sufficient amount of layer was deposited. 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.

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 50 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 40 C [14].

Calibration of Biosensor: The calibration of modified biosensor was studied with 10 mM Urea solution, by dissolving 3.003 gm Urea in 100 ml of water. The stock solution of heavy metal ions [Hg(II)] was prepared in doubly distilled water. An adequate potential was applied and, once a steady-state current was set, a defined amount of urea stock solution was added to the measuring cell. Then, fixed portions of the heavy metal ion stock solution were added consecutively. The addition of heavy metal ion solution resulted in a change in current to the amount of ions added. Enzyme electrodes were conditioned in a stirred phosphate buffer solution for 5 min between each calibration setting [15].

Storage stability study: The stability of the enzyme in the immobilized state was studied by measuring the activity up to 8 days (one set of measurements per day) for both immobilized urease stored in phosphate buffer pH 7.2 at 4oC and that stored at 4oC only. The response of the sensor for 50mM urea concentration was measured. The same electrode was used repeatedly for all the 8 days. The variation of the response current for 50mM urea concentration was recorded [16].

III. RESULT AND DISCUSSION

A. Electrochemical Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) analysis was performed to evaluate the charge transfer character of pure PANI, PANI/ZnO(15%) and PANI/ZnO(15%) + Urease nanocomposite. Figure 1 shows the Nyquist plots obtained in this study. The semi-circle in the high frequency region is observed. This might be due to blocking the ionic exchange at the electrode/electrolyte interface. The straight line in the low frequency is due to the Warburg diffusion impedance. The magnitude of diameter of the semicircle determines nature of charge transfer in the electrochemical system [17]. The impedance curves show a single semicircle in the high frequency region and a straight line in the low frequency region. The diameter of the semicircles is differed from each other. The semi-circle of the PANI/ZnO (15%) is smaller than that of the pure PANI, which suggest that the PANI/ZnO(15%) has a lower electrochemical charge transfer resistance. Thus the charge transfer in the PANI/ZnO(15%) - electrolyte is much faster than that of the pure PANI. The Nyquist plot of the PANI/ZnO(15%) + Urease composite shows well-defined frequency-dependent semicircle impedance curves over high frequencies, followed by straight lines. The linear part in the plot is attributed to the polarization resistance of the electrode. Further, the smaller semicircle observed is an indication of a faster electron transfer rate [18]. It is evident from figure that the PANI/ZnO showed behavior typical of any redox species occurring because of the presence of Urease [19].

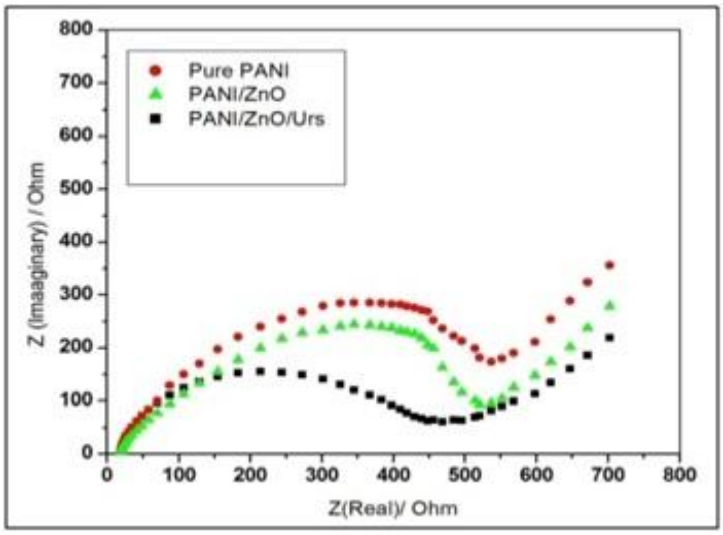

Figure 1: Nyquist Plot for PANI/ZnO/ Urease electrode.

B. Scanning Electron Microscopy (SEM)

(i) SEM Image of Nanostructure ZnO

Figure 2 shows the Scanning Electron Microscope (SEM) images of nanostructured ZnO respectively. It is found that the approximate size of the prepared nanostructured ZnO is in between 100 nm to 150 nm. [20]; [21].

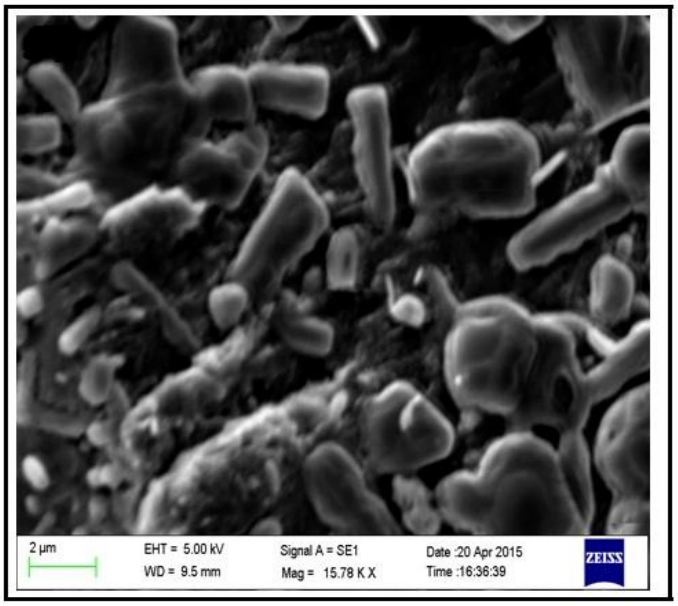

Figure 2: SEM image of Nanostructured ZnO.

(ii) SEM Image of PANI/ZnO/ Urease biosensor

Figure 3 show SEM images of (a) Urease (b) Pure PANI (c) PANI/ ZnO and PANI/ZnO/ Urease. It is observed that ZnO had a strong effect on PANI morphology and showed a transformation in morphology from typical agglomerated form of PANI to particles with high porosity [22]; [23]. Figure (d) shows the entrapment of urease globular structure in to the prepared PANI/ZnO matrix. Thus with the incorporation of ZnO in PANI matrix can increase the porosity of nanocomposites which will fairly help for formation of immobilization of urease for the biosensor applications [24].

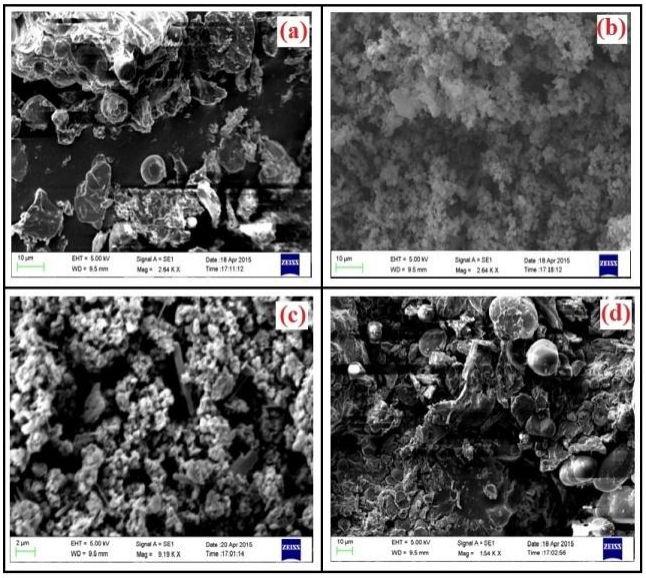

Figure 3: SEM image **(a)** Urease **(b)** Pure PANI **(c)** PANI/ZnO **(d)** PANI/ZnO/Urease.

C. Fourier Transform Infrared (FTIR) Spectroscopy

In order to find the nature of bonding we studied FTIR spectrum of PANI/ZnO nanocomposites. Figure 4 represents FTIR spectra of PANI/ZnO nanocomposites. The peak at 1492cm^{-1} are attributed to characteristics c=c stretching. The broad peak at 1143cm^{-1} which is described by quinonoid as the electronic like band is associated with the vibration mode of N=Q=N (Q refer to the quinine type ring). The strong band at 1139 cm^{-1} is the characteristics peak of PANI. For PANI/ZnO composites corresponding peaks of pure PANI at 798.7 cm^{-1} shifted to 794 cm^{-1}, 1121.8 cm^{-1} shifted to 1136 cm^{-1}, 1472.4 cm^{-1} shifted to 1490.2 cm^{-1}. The shift may be described due to the formation of hydrogen bonding between ZnO and NH group of PANI on the surface of ZnO particles. Which is reported in the literature by many researcher [25]; [26].

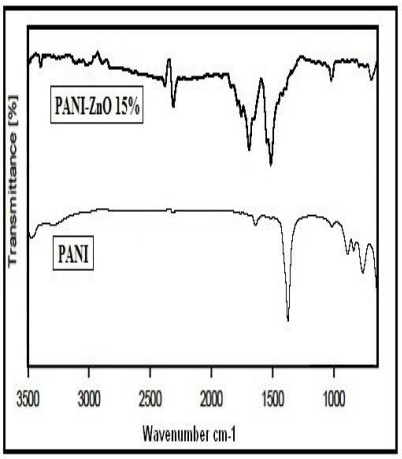

Figure 4: Relative IR peaks of PANI and PANI/ZnO nanocomposite

D. X-Ray Diffraction (XRD)

Figure 5 represents X-rays Diffraction Pattern of PANI/ZnO nanocomposites of (15%), PANI and ZnO. It is observed that PANI is amorphous in nature. The spectrum of prepared ZnO shows sharp peak of Bragg angle 2θ value of 31.2, 34.7, 47.9, 56.9, 68.2 corresponding to [002], [101], [102], [110] and [112] diffraction plane respectively. [JCPDS- FILE NO. 751526]. The grain size the nanoparticle of ZnO was determined using Scherer formula: is around of 65nm. For 15% ZnO composites the XRD spectrum shows peak due to PANI as well as ZnO. Thus PANI undergoes interfacial interaction with ZnO [27].

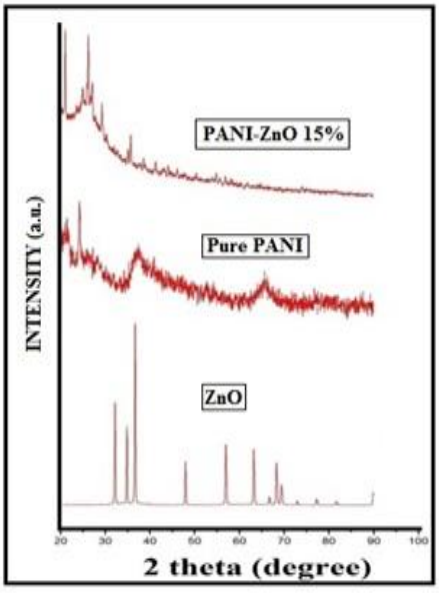

Figure 5: X-RD pattern of PANI/ ZnO nanocomposites

E. Determination of Kinetic Parameters

(i) Calibration curves

The calibration curve of PANI/ZnO/Urease biosensor as shown in Figure 6 was calculated by taking the maximum current reading for each concentration and plotting current value versus Urea concentration. From the calibration curve it is observed that the biosensor shows good linearity in the range of 10 - 50 mM of Urea concentration. The detection limit is calculated according to equation (3 x SD/m), where SD is the standard deviation with blank signal (R^2) and m is the slope of the calibration curve [28]. Using the formula, the minimum detection limit is found to be 4.953 mM/l. Thus the constructed biosensor shows low detection limit for urea which may be attributed to incorporation of nanostructured ZnO for immobilizing urease, which provides larger surface to volume ratio. The linear regression equation was I(mA) = 0.599 x [Urea Conc.] + 7.235 with correlation coefficient (R^2) of 0.989. The sensitivity is the slope of calibration curve, which is found to be 0.599 mA/mM (Table 1).

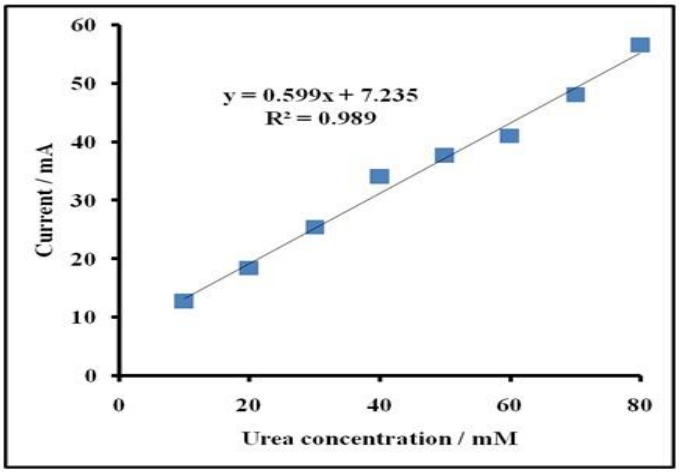

Figure 6: Calibration curve of PANI/ZnO/Urease Biosensor.

Table 1: Analytical characteristics and regression parameters of biosensors for the detection of urea.

Biosensor	Linear Range	Sensitivity (S)	Correlation coeff. (R^2)	Limit of Detection (LOD)
PANI/ZnO/Urease	10 -50 mM	0.599 mA/mM	0.989	4.953 mM/l

(ii) Lineweaver–Burk plots

Lineweaver–Burk plot between 1/current (mA) and 1/Urea concentration (mM) is shown in Figure 7. The value of Michaelis–Menten constant (K´m) for Urease was obtained as K´m =0.018 mM and maximum current (Imax) was found to be 83.33 mA. The literature values of the Michaelis-Menten constant for urease lie between 0.012 mM and 1.30 mM. Therefore the KM value of 0.018 is within the range of the literature values.

This K´m value is lower than that for free enzyme (1.30 mM) indicating increased affinity of enzyme toward Urea after immobilization [29], which might be due to enhanced diffusion of Urea through *PANI/ZnO/Urease* surface.

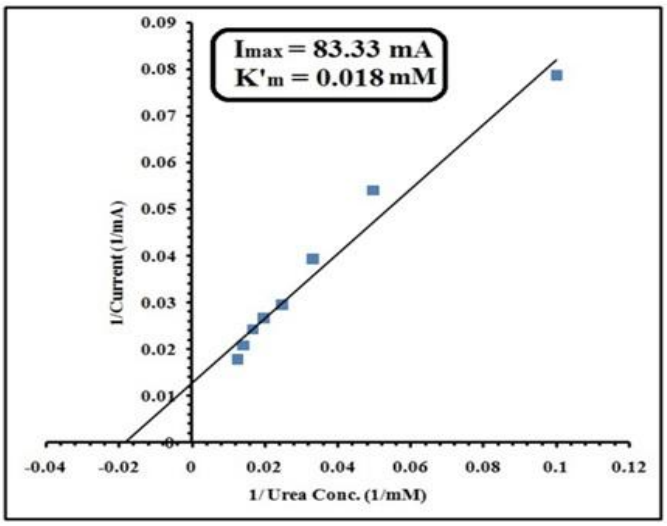

Figure 7: Lineweaver–Burk plot of PANI/ZnO/Urease Biosensor

F. Determination of Kinetic Parameters

Figure 8 shows the response of PANI/ZnO/Urease Biosensor as a function of storage time in the presence of Urea 10 mM in phosphate buffer (0.1 M, pH 7.2). The biosensor was tested for stability over 10 days. When not in use, the electrodes were stored at 4°C. It can be seen that there is an initial sharp decline in response followed by a gradual decrease. After about 10 days the sensor response is less significant and thus cannot be used for Urea determination.

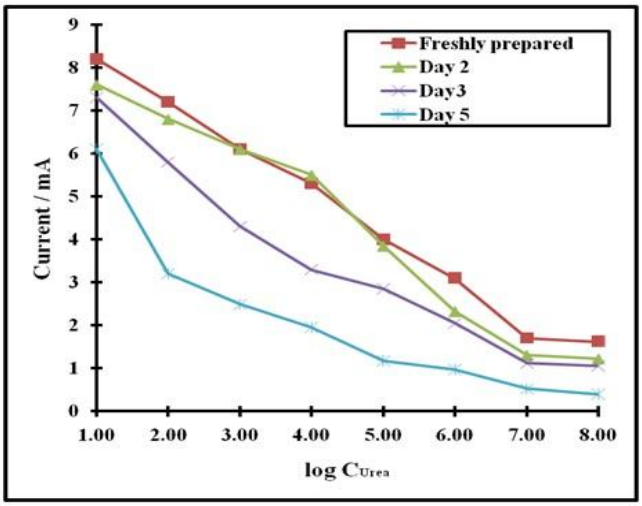

Figure 7: Storage stability of PANI/ZnO/Urease Biosensor.

IV. CONCLUSIONS

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 calibration curve of PANI/ZnO/Urease biosensor indicates that the biosensor has good linearity in the range of 10 - 50 mM of Urea concentration. The minimum detection limit is found to be 4.953 mM/l. Michaelis–Menten constant (K´m) for immobilized Urease was obtained as 0.018 mM and maximum current (Imax) was found to be 83.33 mA. This K´m value is lower than that for free enzyme (1.30 mM) indicating increased affinity of enzyme toward Urea after immobilization, which might be due to enhanced diffusion of Urea through surface.

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