

Comparative Study of Chronoamperometry of PANI/ZnO/Urease and PANI/MnO₂/Urease Biosensors

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Abstract: Polyaniline (PANI) based electrochemically synthesized PANI/ZnO/Urease and PANI/MnO₂/Urease biosensors have been prepared. The stainless-steel transducer was used for electrodeposition using potentiostat. Chronoamperometric response of as-synthesized PANI/ZnO/Urease and PANI/MnO₂/Urease biosensors in potential range 0.2 to 0.6 volt vs. reference electrode for time interval of 100 Sec in PBS of pH 7 was carried out. The immobilization of urease on modified PANI/ZnO (15%) film, results in getting larger saturation current in 10 sec suggests that the immobilized enzyme by physical adsorption method is well entrapped in PANI/ZnO matrix and show lesser degradation of the Urease. PANI/MnO₂ matrix, show degradation of the Urease on account of the less stable curve. The decay of the saturation current in PANI/MnO₂/Urease suggests the predominance of the degradation of enzyme layer over electron transfer at the electrode surface. PANI/ZnO matrix is found more suitable for the entrapment of Urease compared to the PANI/MnO₂ matrix.

Keywords: Polyaniline, ZnO, MnO₂, Chronoamperometry, Urease, biosensor

I. INTRODUCTION

In last two decades, the development of biosensor has oriented much attention of the scientists from different research fields such as food industry, bio-industry, medicine and environmental control because of their ability to give reliable and fast response to the presence of a specific compound without disturbing the system [1]. The electrochemical method of measuring current at a constant potential is called as amperometric technique. Many literatures reported Urease (Urs) as biological element for the development of urea biosensors [2, 3], which are usually applied in environmental tests and medical diagnostics. Amongst the various conducting polymers, Polyaniline [PANI] has rapidly become the subject of considerable interest for physicists, chemists and material scientists [4]. Now a day preparation of PANI by electrochemical polymerization is most widely used technique in industries and laboratory research work. The advantages of electrochemical process over the conventional chemical process are absence of catalyst, direct coating of the doped conducting polymer onto the electrode surface, and easy control of the film thickness by just varying the current through electrolyte [5]. Nano structured oxides of metals such as Zinc, Iron, Cerium, Tin, Zirconium, Titanium, and Manganese have been found to exhibit interesting nano morphological, functional biocompatible, non-toxic and catalytic properties [6]. Among the various NSMO based immobilizing matrices nanostructured oxides of Zinc [7] and Manganese [8], have exceptional optical and electrical properties due to electron and phonon confinement, high surface-to-volume ratios, modified surface work function, high surface reaction activity, high catalytic efficiency and strong adsorption ability.

The transducer is a key component in the construction of biosensor. A certain number of studies have been reported for the use of interdigitated thin film electrodes [9], Titanium, chromium, Aluminum, Platinum, mild steel [10-11] etc as a transducer element. But, Titanium, chromium and Aluminum 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 deposit on stainless steel by electro polymerization [12]. The Electropolymerized layer on stainless steel electrode

immobilized with the enzyme gave water-insoluble, transparent film, with a high enzyme activity. Chronoamperometry is the technique in which we measure current ('amp-') as a function of time ('chrono-'). During the Chronoamperometry experiment the potential is stepped from an initial potential E_1 at which no electro modification occurs to a second potential E_2 at which the electrode reaction is complete. Since the current is integrated over relatively longer time intervals, Chronoamperometry gives a better signal to noise ratio in comparison to other amperometry technique[13]. This useful technique gives initial information about nucleation and growth mechanism in a studied system.

In the present work, we have developed two types of electrochemical amperometric biosensor namely 'PANI/ZnO/Urease' and 'PANI/MnO₂/Urease' by immobilizing an enzyme 'Urease', to the transducer surface 'Stainless steel' with different weight percentage of nanomaterials such as 5% 10% and 15% by the method of electropolymerization. The chronoamperometry of individual biosensor was carried and compared to investigate the relative response of these biosensors. The Chronoamperometric response of the Bare Steel electrode, Pure PANI coated electrode, 'PANI/ZnO(15%)+Urease' and 'PANI/MnO₂(15%)+Urease' was also studied.

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 [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 85° C. then, the nanocrystalline ZnO powder was filtered and oven dried.

2.3 Synthesis of Nano-sized MnO₂

The solution of 0.4M aniline was prepared in 100 ml of 1M H₂SO₄. The electrode position 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- MnO₂ nanocomposites with different weight percentage were deposited on Stainless Steel surface by linear swiping the voltage in range -200 mV to +1100 mV at 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.

2.4 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.

2.5 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.

2.6 Chronoamperometry Technique

Potentiostat/Galvanostat (Model CHI600D Electrochemical Analyzer) was used to record Chronoamperometry Curves of the conducting polymers and their nanocomposites under study. The fabricated PANI/ZnO/Urease and PANI/MnO₂/Urease electrodes with different weight percentage of nanostructure (5%, 10% and 15%) were investigated in phosphate buffer solution (PBS). Chronoamperometric study was performed by immersing modified SS electrodes in PBS with constant stirring at 100 rpm [15]. Chronoamperometric scan was taken by biasing the electrode at 0.85 V vs Ag/AgCl reference electrode.

III. RESULT AND DISCUSSION

3.1 Chronoamperometry of PANI/ZnO/Urease Electrode

Figure 3.1 illustrates the chronoamperometric response of the Pure PANI, PANI/ZnO (5%, 10% and 15%) and PANI/ZnO/Urease (15%) electrodes in the potential range 0.2 to 0.6 volt vs. reference electrode for time interval of 100 Sec in PBS of pH 7. The current of PANI coated electrode without ZnO and with 5% ZnO increases up to approximately 10s and then shows saturation current for further increase in time. The lower saturation current might be due to lesser weight percentage of ZnO which in turn suggest poor electron transfer at the electrode surface. In contrast, the current for PANI/ZnO (10% and 15%) coated electrode saturate at significantly larger value. This might be due to higher surface to volume ratio as well as relaxation by mass transport of increased ZnO concentration. The increase in the current suggests the predominance of the fast electron transfer over the degradation of enzyme layer in to the electrolyte, whereas the decrease means the predominance of the degradation over the fast electron transfer [16].

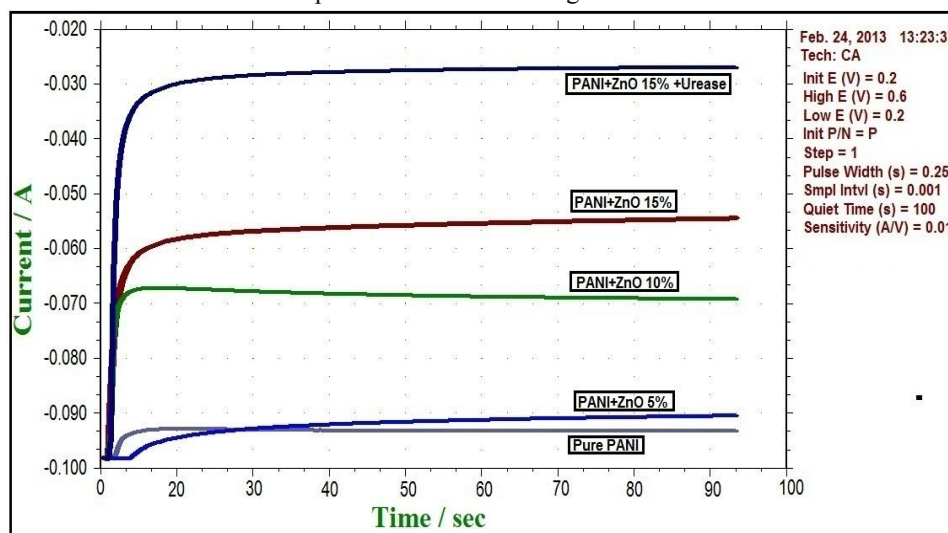


Figure 3.1 Chronoamperometry of PANI/ZnO/Urease electrode.

3.2 Chronoamperometry of PANI/MnO₂/Urease electrode

Figure 3.2 illustrates the chronoamperometric response of the Pure PANI, PANI/MnO₂ (5%, 10% and 15%) and PANI/MnO₂/Urease (15%) electrodes in the potential range 0.2 to 0.6 volt vs. reference electrode for time interval of 100 Sec in PBS of pH 7. We obtained the steady-state conditions from chronoamperometric curves at different weight

percentage of nanostructured MnO_2 . The current of PANI coated electrode without MnO_2 and with MnO_2 increases up to approximately 10s and then shows saturation current for further increase in time. The nature of the all curves for three weight percentage composition (5%, 10% and 15%) shows similar behavior. Thus increase in the MnO_2 weight percentage in the PANI matrix does not attribute towards the larger saturation current and hence does not guarantee for the fast electron transfer at the modified electrode surface [17].

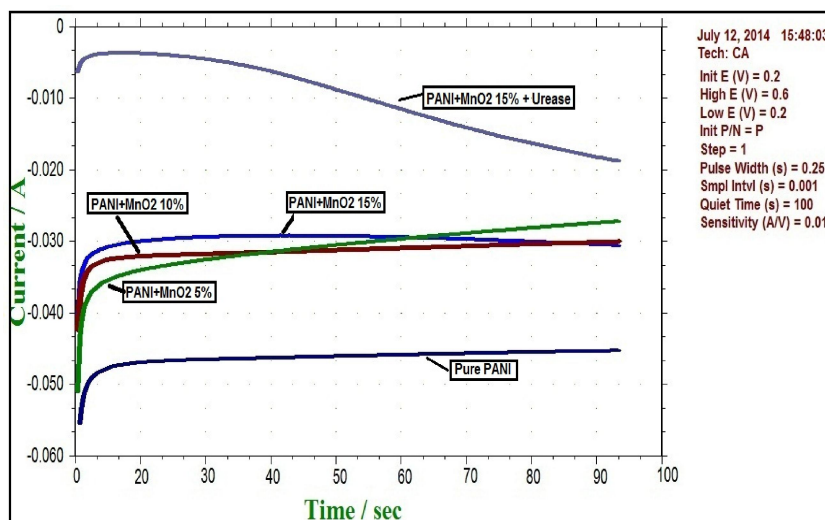


Figure 3.2Chronoamperometry of PANI/ MnO_2 /Urease electrode.

3.3 Comparison of Chronoamperometry of PANI/ ZnO /Urease and PANI/ MnO_2 /Urease

Figure 3.3 illustrates the chronoamperometric response of the Bare Steel electrode, Pure PANI coated electrode, 'PANI/ ZnO (15%)+Urease' and 'PANI/ MnO_2 (15%)+Urease' electrodes in the potential range 0.2 to 0.6 volt vs. reference electrode for time interval of 100 Sec in PBS of pH 7. It is observed that the current increase in case of PANI coated steel electrode as compared to bare steel electrode. Also the saturation current increases due to incorporation of nanostructured ZnO and MnO_2 in the PANI matrix. The decay of the saturation current in PANI/ MnO_2 (15%) + Urease electrodes suggests the predominance of the degradation of enzyme layer over electron transfer at the electrode surface. Thus the PANI/ ZnO matrix is more suitable for the entrapment of Urease as compared to the PANI/ MnO_2 matrix [18].

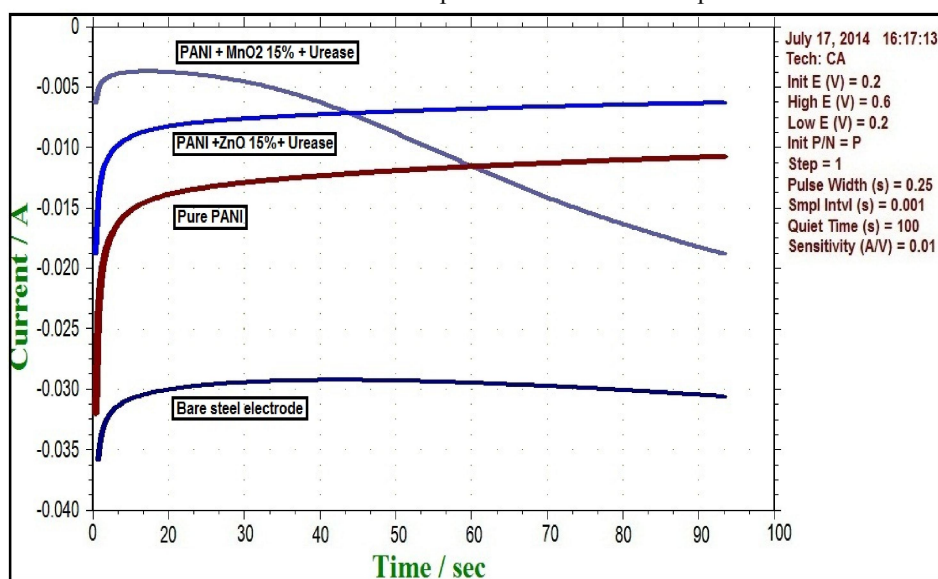


Figure 3.3Comparison of Chronoamperometry of PANI/ ZnO / Urease and PANI/ MnO_2 / Urease.

IV. CONCLUSION

The present investigation shows that the immobilization of urease on both modified films PANI/ ZnO (15%) and PANI/ MnO₂ (15%) gives larger saturation current in the time of 10 sec. This suggests that the immobilized enzyme by physical adsorption method is well entrapped in to the PANI/ZnO as well as PANI/ MnO₂ matrix and it will not show degradation of the Urease from the both electrode surfaces. But the curve obtained in case of PANI/ MnO₂ is not stable for long time. Thus the PANI/ MnO₂ matrix show early degradation of the Urease as compared to PANI/ZnO matrix. This might be due to low Isoelectric point (IEP) of MnO₂ (4 to 5) compared to ZnO (9 to 9.5). The increase in the MnO₂ weight percentage in the PANI matrix does not attribute towards the larger saturation current and hence does not guarantee for the fast electron transfer at the modified electrode surface. The chronoamperometric response of the Bare Steel electrode, Pure PANI coated electrode, 'PANI/ZnO (15%)+Urease' and 'PANI/MnO₂ (15%)+Urease' electrodes shows the current increase in case of PANI coated steel electrode as compared to bare steel electrode. Also the saturation current increases due to incorporation of nanostructured ZnO and MnO₂ in the PANI matrix. The decay of the saturation current in 'PANI/MnO₂ (15%)+Urease' electrodes suggests the predominance of the degradation of enzyme layer over electron transfer at the electrode surface. Thus it is concluded that the PANI/ZnO matrix is more suitable for the entrapment of Urease as compared to the PANI/MnO₂ matrix.

REFERENCES

- [1]. P. Leonard, S. Hearty, J. Brennan, L. Dunnea, J. Quinn, T. Chakraborty, R. O'Kennedy, Advances in biosensors for detection of pathogens in food and water, *Enzyme and Microbial Technology*, 32, (2003), 3–13.
- [2]. M. Massafra, S. Cordoba de Torresi, Urea amperometric biosensors based on a multifunctional bipolymeric layer: Comparing enzyme immobilization methods, *Sensors and Actuators B*, 137, (2009), 476–482.
- [3]. S. Chawla, R. Rawal, S. Sharma, C. S. Pundir, An amperometric biosensor based on laccase immobilized onto nickel nanoparticles/carboxylated multiwalled carbon nanotubes/polyaniline modified gold electrode for determination of phenolic content in fruit juices, *Biochemical Engineering Journal*, 68, (2012), 76–84.
- [4]. N. Bicak, B. Karagoz, Polymerization of Aniline by Copper-Catalyzed Air Oxidation, *Journal of Polymer Science: Part A: Polymer Chemistry*, 44, (2006), 6025–6031.
- [5]. R. Ansari, M. B. Keivani, Polyaniline Conducting Electroactive Polymers: Thermal and Environmental Stability Studies, *E-Journal of Chemistry*, 3, 4, (2006), 202–217.
- [6]. P. R. Solanki, A. Kaushik, V. V. Agrawal, B. D. Malhotra, Nanostructured metal oxide-based biosensors, *NPG Asia Mater.*, 3, 1, (2011), 17–24.
- [7]. S. Ghasemi, M. F. Mousavi, M. Shamsipur, Enhancement of electron transfer kinetics on a polyaniline-modified electrode in the presence of anionic dopants, *J. Solid State Electrochem*, 12, (2008), 259–269.
- [8]. L. Chen, Z. Song, G. Liu, J. Qiu, C. Yu, J. Qin, L. Ma, F. Tian, W. Liu, Synthesis and electrochemical performance of polyaniline–MnO₂ nanowire composites for supercapacitors, *Journal of Physics and Chemistry of Solids*, 74, (2013), 360–365.
- [9]. N. Jaffrezic-Renault, S. V. Dzyadevych, Conductometric Microbiosensors for Environmental Monitoring, *Sensors*, 8, (2008), 2569–2588.
- [10]. F. Branzoi, V. Branzoi and A. Musina, Amperometric urea biosensor based on platinum electrode modified with a nanocomposite film, *Surf. Interface Anal.*, 44, (2012), 895–898.
- [11]. J. L. Camalet, J.C. Lacroix, T. D. Nguyen, S. Aeiach, M.C. Pham, J. Petitjean, P.-C. Lacaze, Aniline electropolymerization on platinum and mild steel from neutral aqueous media, *Journal of Electroanalytical Chemistry*, 485, (2000), 13–20.
- [12]. Y. Obaid, E. H. El-Mossalamy, S. A. Al-Thabaiti, I. S. El-Hallag, A. A. Hermas, A. M. Asiri, Electrodeposition and Characterization of Polyaniline on Stainless Steel Surface via Cyclic, Convulsive Voltammetry and SEM in Aqueous Acidic Solutions, *Int. J. Electrochem. Sci.*, 9, (2014), 1003–1015.

- [13]. F. Branzoi, V. Branzoi, A. Musina, Amperometric urea biosensor based on platinum electrode modified with a nanocomposite film, *Surf. Interface Anal.*, 44, (2012), 895–898.
- [14]. B. Lakard, G. Herlem, S. Lakard, A. Antoniou, B. Fahys, Urea potentiometric biosensor based on modified electrodes with urease immobilized on polyethylenimine films, *Biosensors and Bioelectronics*, 19, (2004), 1641–1647.
- [15]. J. Singh, S. K. Mittal, *Chlorella sp.* based biosensor for selective determination of mercury in presence of silver ions, *Sensors and Actuators B*, 165, (2012), 48-52.
- [16]. M. Moyo, J. O. Okonkwo, N. M. Agyei, Recent Advances in Polymeric Materials Used as Electron Mediators and Immobilizing Matrices in Developing Enzyme Electrodes, *Sensors*, 12, (2012), 923-953.
- [17]. A. P. Mahajan, S. B. Kondawar, R. P. Mahore, B. H. Meshram, P. D. Virutkar, Polyaniline/MnO₂ nanocomposites based stainless steel electrode modified enzymatic urease biosensor, *Procedia Materials Science*, 10, (2015), 699-705.
- [18]. K. Aoki, S. Tano, Simultaneous occurrence of polymerization and decomposition of polyaniline films, *Electrochimica Acta*, 50, (2005), 1491-1496.