HRP modified pulse laser ablated ZnO nanostructure for H_2O_2 sensing

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Received: 31 March 2016, Revised: 07 August 2016 and Accepted: 03 September 2016

DOI: 10.5185/amp.2016/209
www.vbripress.com/amp

Abstract

Biosensor for the detection of hydrogen peroxide (H_2O_2) has been prepared by immobilizing horseradish peroxidase (HRP) enzyme using physical adsorption technique on zinc oxide (ZnO) nanostructures. The (002) oriented ZnO nanostructures as confirmed by X-ray diffraction, were successfully grown on indium tin oxide (ITO) coated glass substrate by pulsed laser ablation (PLA) without using any catalyst. The Nafion solution was added onto HRP/ ZnO/ ITO bio-electrode to form a tight membrane on the surface before carrying out bio-sensing measurements by electrochemical analyzer. The electrochemical studies reveal that the prepared bio-electrode HRP/ZnO/ITO is highly sensitive to the detection of H_2O_2 over a wide range of concentration with a linear range from 2.5 μM to 100 μM with the limit of detection 0.2 μM and sensitivity of 0.034 μA/ μM cm^2. The higher sensitivity attributed to larger surface area of ZnO nanostructure for effective loading of HRP besides its high electron communication capability. A relatively low value of the enzyme's kinetic parameter (Michaelis-Menten constant, K_m) of 0.166 μM indicates enhanced enzyme affinity of HRP to H_2O_2. The reported biosensor may be useful for various applications in bio-sensing, clinical, food and beverage industry. Copyright © 2016 VBRI Press.

Keywords: ZnO, pulsed laser ablation/deposition (PLA/PLD), HRP, H_2O_2, biosensor.

Introduction

Sensitive and accurate determination of hydrogen peroxide (H_2O_2) is of practical importance in many fields such as biochemistry, chemical, environmental analysis, medicine, food process, biomedical and pharmaceutical industries [**1**]. In addition, use of H_2O_2 as an antibacterial agent makes it important substance in the food and beverage industries [**2**]. It has also been noted that H_2O_2 is necessary for the metabolism of proteins, carbohydrates, fats, vitamins and minerals. H_2O_2 can be very effective in regulating blood sugar and cellular energy production in the body [**3**]. H_2O_2 has been recognized as one of the most stable reactive oxygen species in the body, which has a significant role in development and progression of different neurodegenerative diseases [**4**]. These applications have raised extensive demands for establishing protocols for H_2O_2 detection. Therefore, the accurate detection and constant monitoring of H_2O_2 is essential and important not only in the context of industry but for the human body also.

A number of techniques have been used for the detection of hydrogen peroxide (H_2O_2), for example, titrimetry, spectrometry, photometry, fluorescence, chemiluminescence and electrochemistry [**5,6**]. Among these, electrochemical technique is widely used because of its simplicity in use, cost effective, high sensitivity and better selectivity [**7**]. Electrochemistry is the study of the chemical response of a system to an electrical stimulation. Electrochemistry studies the reduction and oxidation (redox reactions) and can provide information about the concentration, kinetics, reaction mechanisms, chemical status and other behavior of a species in solution. A three electrode system is generally used in electrochemical experiment – the working electrode, the reference electrode and the counter (or auxiliary) electrode.

In electrochemistry, electrochemical biosensors are widely used for sensing glucose, cholesterol, urea, hydrogen peroxide etc. For developing a biosensor, choice of the suitable matrix over which enzyme can be immobilized without much loss of its bioactivity is very important. Thus, developing an efficient matrix having a compatible microenvironment for the bio-molecules with good electron transfer capability is desirable to realize an efficient biosensor with enhanced sensitivity and rapid response. A variety of matrices such as metals, metal oxides, composites, nanoparticles, conducting polymers have been used as a platform for immobilization of bio-molecule.

Recently, zinc oxide (ZnO) nanostructures based electrochemical biosensors have been attracted considerable attention due to their ability to promote

faster electron transfer between the electrode and the active site of desired enzyme, high isoelectric point (IEP) of 9.5 and ease of fabrication [**8-11**]. Zhiwei *et al.* [**12**] have reported on sensing of glucose, hydrogen peroxide, phenol, cholesterol, *etc.* using ZnO nanostructures with an emphasis on ZnO fabrication techniques, enzyme immobilization methods, and biosensor performance. State of electrochemical sensing of hydrogen peroxide using different metal (Ag, Pt, Pd, Cu etc), bimetallic nanoparticles and other metals have been reported by Shihong *et al.* [**13**]. Waxberry-liked ZnO nanorods synthesized by wet chemical method have been reported for H_2O_2 sensing by Cao *et al.* [**14**]. In this article, horseradish peroxidase (HRP) enzyme was immobilized on the ZnO nanorods for preparing nano-ZnO/HRP electrode. This biosensor showed a wider linear range, from 0.l5 to 15 mM, and a detection limit of 0.115 μM [**14**]. In another study, Liu *et al*. [**15**] decorated ZnO nanoarrays with carbon and reported high sensitivity (237.8 $\mu A/cm^2$ mM) and fast response (4s) in H_2O_2 sensing [**15**]. Another biosensor based on nanoporous ZnO/chitosan exhibiting a sensitivity of 43.8 $\mu A/cm^2$ mM has been reported by Yang *et al.* [**16**]. In another example, ZnO and chitosan composite based biosensor in which gold solution used as immobilization platform has been reported [**17**]. This biosensor had a fast response to H_2O_2 and excellent linear relationships from 0.19 μM to 1.73 mM. ZnO nanorods array grown on indium tin oxide (ITO) coated glass substrate based biosensor had been developed with the low detection limit of 2.0×10^{-13} mol L^{-1} by Wang *et al.* [**18**] and Xiang *et al.* [**19**] developed flowerlike ZnO–gold nanoparticles (GNPs)–Nafion nanocomposite modified glassy carbon electrode using wet chemical method. The nanocomposite helped towards the direct electron transfer of HRP enzyme immobilized in the film effectively. The device reportedly had shown an enhanced electrocatalytic activity towards the reduction of H_2O_2. The biosensor demonstrated that nanocomposite film provided a favorable microenvironment for the enzyme to retain its activity by showing a linear increase of catalyst current to H_2O_2 concentration over a wide range of 1.5×10^{-5} to 1.1×10^{-3} M and the detection limit of 9.0×10^{-6} M. Flower-like hierarchical ZnO nanostructures functionalized further with Au and Ag nanoparticles have been synthesized by Hussain *et al.* [**20**]. These nanostructures showed good amperometric response to H_2O_2, with a linear range from 1 to 20 μM, and detection limit of 2.5 μM. The sensor shows high and sensitivity of 50.8 μA cm^{-2} μM^{-1}.

Considering the significance of ZnO nanostructures as demonstrated in literature towards H_2O_2 sensing, it is scientifically important to grow these nanostructures without using any catalyst onto ITO coated glass substrate. The ZnO nanostructures oriented along (002) presented in this paper has been directly grown on ITO coated glass substrate using pulsed laser ablation/deposition (PLA/PLD) technique. HRP enzyme immobilized on ZnO/ITO nanostructure for preparing HRP/ ZnO/ ITO bio-electrode used in electrochemical studies for H_2O_2 sensing. The electrochemical response as a function of H_2O_2 concentration has been studied in detail using HRP/ZnO/ITO biosensor. The synthesized well oriented ZnO nanostructures may be useful for the possible device applications [**21**].

Experimental

Materials

Zinc oxide (99.99%, Sigma Alderich USA) was used for preparing target required for the growth of ZnO nanostructures on ITO coated glass (resistivity: 70-100 Ω/sq, Sigma Aldrich, USA) substrate using PLD. Horseradish peroxidase (250- 300 unit/ mg) and Nafion were obtained from Sigma Aldrich, life sciences, USA. Hydrogen peroxide (Thomas baker chemical Pvt. limited Mumbai, India) used as sensing element. All the solutions were prepared in Milli-Q ultrapure water.

Material synthesis / Reactions

The ZnO target used in PLD was prepared in house by sintering a ZnO pellet at 1000 ºC for 4h in the air. Further, second harmonic (λ = 532 nm) of Nd- YAG laser (Thunder one Quanta systems, DNA laser technologies, Italy) with energy fluence 3.0 J/cm^2 was used to ablate the ZnO target at 0.2 mbar oxygen pressure in PLD at our laboratory. The substrate temperature was maintained at 500 °C during deposition.

To prepare the biosensor, first the HRP enzyme was immobilized on a ZnO matrix by physical adsorption technique. HRP (1mg/ml) has been prepared in phosphate buffer solution (PBS) (pH 7.0). 10 μl solution of HRP has been uniformly spread over the desired area of ZnO/ ITO electrode. The HRP/ZnO/ITO bio-electrode was then kept at room temperature for 4h. Finally, the bio-electrode was washed with phosphate buffer to remove any unbound enzyme from electrode surface. Thereafter, 10 μl Nafion solution was added over HRP/ ZnO/ ITO bio-electrode to form a tight membrane on the surface and allowed to dry at room temperature for 2h. The modified electrode was rinsed with PBS and stored at 4ºC when not in use.

Characterizations

The orientation and crystalline properties of the ZnO nanostructures were characterized using high resolution X-ray diffraction (HR-XRD, Smart lab 3kW, Rigaku, Japan) with Cu K_α radiation (λ=1.54Å). Surface morphology was investigated using atomic force microscopy (AFM Agilent 5500, Agilent technologies, USA). Optical properties of

ZnO nanostructure were studied using spectroscopic ellipsometer VASE (J.A. Wollam Co. Inc., USA) and photoluminescence spectrophotometer (PL, LS 45, Perkin Elmer, USA). The PL spectrum was recorded with excitation at 325 nm for detecting PL peaks in the range 350- 600 nm (3.54- 2.06 eV). The transport properties were studied for ZnO nanostructures using scanning tunneling microscopy (STM, Nano Rev, Quazar technologies, India).

The electrochemical experiment was performed at room temperature using AutoLab Potentiostat /galvanostat (PGSTAT 101, Metrohm Autolab B.V., The Netherlands) with a conventional three-electrode cell configuration having Ag/AgCl electrode as a reference electrode, Nafion/HRP/ZnO /ITO as the working electrode and platinum wire as a counter electrode in PBS (pH 7.0, 50mM) solution containing 5mM $K_3[Fe(CN)_6]$ as a redox couple. The characterizations have been carried out in our laboratory (Centre for Interdisciplinary Research) at MNNIT Allahabad.

Results and discussion

Structural and morphological studies

The XRD data for ZnO nanostructures deposited on ITO glass and bare ITO glass is shown in **Fig. 1(a).** The XRD data of bare ITO substrate is also shown in figure for comparison. It could be noted from the figure that ZnO nanostructure shows a dominant (*002*) reflection peak representing a preferential growth along c axis normal to the ITO coated glass substrate [*International Centre for Diffraction Data* (ICDD) 04-016-664]. The XRD data revealed (*00l*) oriented growth of wurtzite ZnO structure. The AFM image [**Fig. 1(b)**] of as- grown ZnO nanostructure reveals the formation of rough microstructure having uniform distributed nano pores. The average rms roughness of the film surface has been found to be about 7 nm.

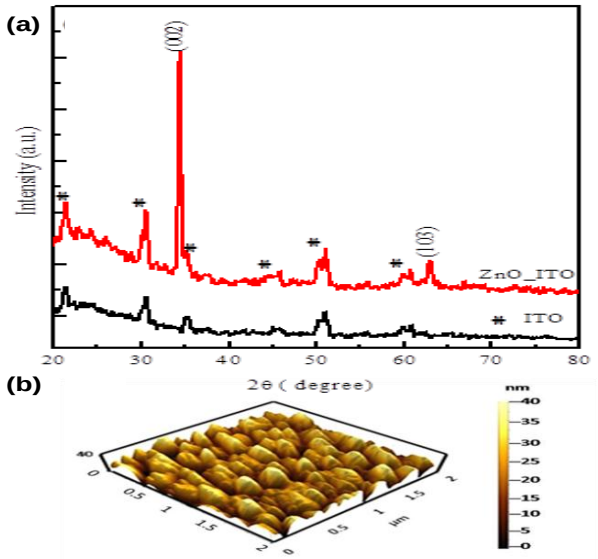

Fig. 1. (a) XRD pattern of ZnO/ITO and ITO (b) AFM image of ZnO on ITO coated glass substrate.

Optical studies

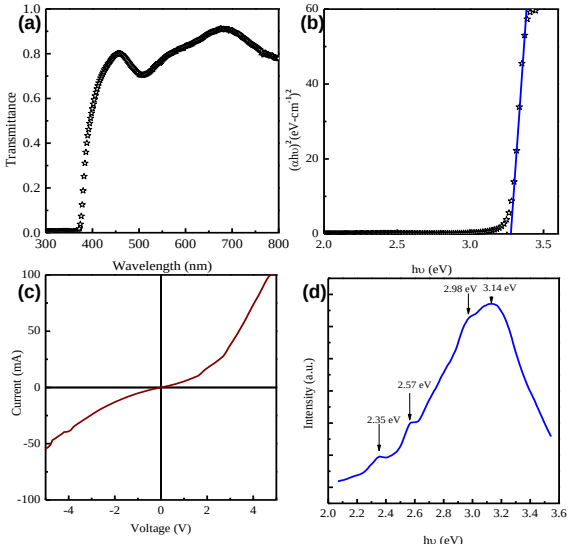

Fig. 2. (a) Transmittance spectra (b) $(\alpha h\nu)^2$ vs. hν plot (c) I-V characteristic curve and (d) PL spectra for ZnO deposited on ITO glass substrate.

Fig. 2(a) shows the room temperature transmittance spectra as a function of wavelength for ZnO. It is observed that ZnO nanostructures exhibit optical transmittance over 83% in the visible region and fundamental adsorption edge were found around 379 nm. The value of the optical band gap was estimated as 3.27 eV using Tauc plot [**Fig. 2(b)**]. Current transport property [Current-voltage, I-V] of ZnO was studied using I- V characteristic curve and shown in **Fig. 2(c)**. The I-V curve, shows a nonlinear behavior somewhat like a diode. The ideality factor was also calculated using the slope of the ln I – V characteristics. The measured value of ideality factor at room temperature for ZnO is 2.56. High value of ideality factor, confirms the non-ideal behavior of the junction. The non-ideal behavior of the junction can be due to non-uniformity schottky contacts because of intermediate states, surface states and defects [**22**].

PL measurements have been carried out to explore the various zinc and oxygen related defects in ZnO nanostructures. PL spectra were recorded at room temperature and shown in **Fig. 2(d)**. It shows a large UV peak centered at around 3.14 eV(~396 nm) followed by low intensity peaks at around 2.98 (~ 416 nm), 2.57 eV (~ 482 nm) and 2.35 eV (~ 2.35 eV). As the optical band gap was found to be around 3.27 eV, the observed PL peak at 3.14 eV may be due to free exciton emission and transition of neutral donor bound excitons emission [**23, 24**]. PL peak at 2.98 (~ 416 nm) may be due to the development of neutral zinc vacancy related emission [**25**]. The blue emission at 2.57 eV may be due to oxygen rich defects [**26**] such as O_i, O_{Zn}, V_{Zn} as ZnO nanostructures are grown under oxygen environment (0.2 mbar). A peak around 2.35 eV corresponds to green emission and indicates the presence of oxygen vacancies. This can be due to an electron transition

from shallow donor level (V_o) to a shallow acceptor level (V_{Zn}) [**27**].

Cyclic voltammetric (CV) studies

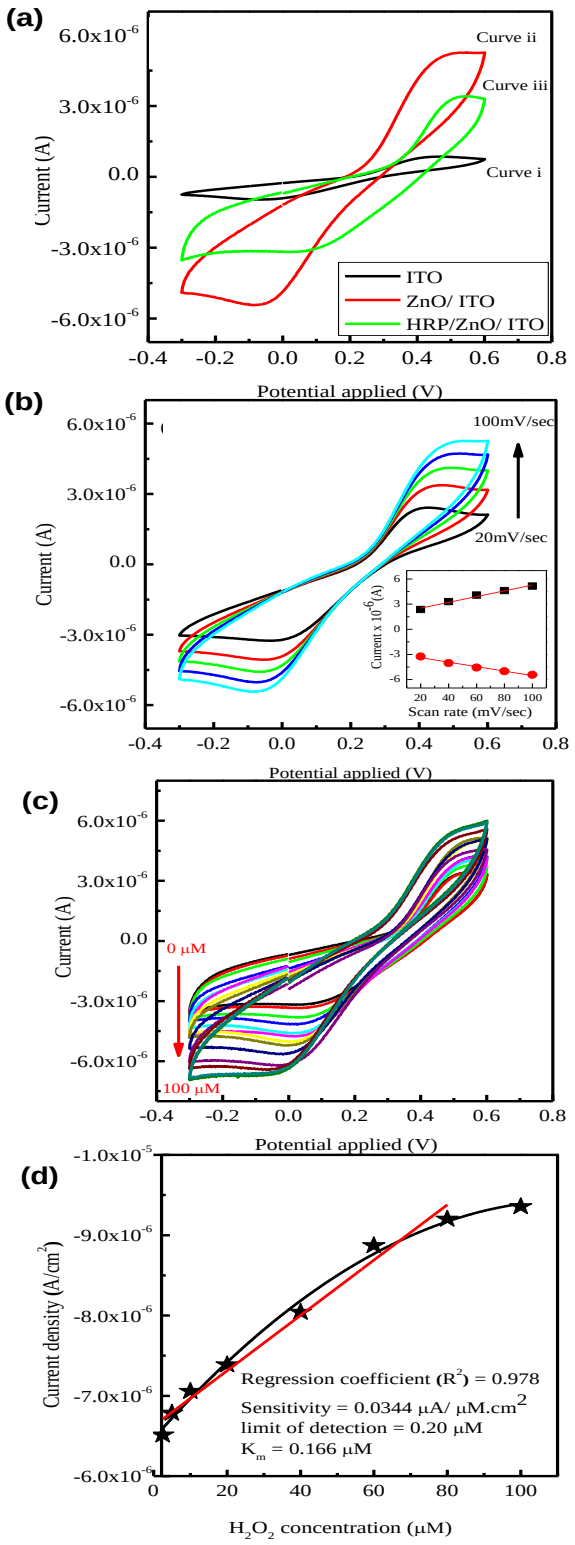

Fig. 3. Cyclic voltammograms of (a) ITO, ZnO/ITO & HRP/ZnO/ITO electrode (b) ZnO/ITO electrode at scan rates of 20, 40, 60, 80, 100 mV/sec; inset showing [variation in current at different scan rates] (c) HRP/ZnO/ITO plate in 0.2 M Phosphate buffer (pH 7.0) at different H_2O_2 conc. from 0µM to 100 µM in 0.2 M Phosphate buffer (pH 7.0) (d) Variation of current density as a function of H_2O_2 concentration.

The enzymatic activity of prepared biosensor was investigated using cyclic voltammetry (CV) in phosphate buffer solution (PBS). **Fig. 3(a)** represents CV of bare ITO, ZnO/ITO, HRP/ZnO/ITO electrodes in PBS (pH 7.0, 50 mM) containing K_3 [Fe $(CN)_6$] as mediator at scan rate of 100mV/sec in the range -0.3 to 0.6 V. The result of CV studies shows that magnitude of reduction current increases due to ZnO/ ITO electrode (curve ii) compared to that of bare ITO electrode (curve i). This may be due to well oriented ZnO having a good electrochemical catalytic property with enhanced charge communication characteristic [**28**]. However, the magnitude of current response decreases after immobilization of HRP on to ZnO/ITO bio electrode (curve iii) which may be because of strong binding of HRP with the ZnO / ITO electrode and insulating characteristics of HRP which possibly decreases the transfer of electron between medium and HRP modified bio-electrode [**29**]. **Fig. 3(b)** exhibits CV investigation of HRP/ZnO/ITO bioelectrode as a function of scan rate from 20 to 100 mV/s. It is observed that the magnitude of both anodic and cathodic current increases linearly with the scan rate (inset), indicating improved electro catalytic behavior of bio electrode. **Fig. 3(c)** shows the electrochemical response of the HRP/ZnO/ITO bio-electrode in PBS containing 5mM K_3 [Fe (CN) $_6$] for varying concentration of H_2O_2. The modified HRP/ZnO/ ITO electrode showed good electro catalytic activity towards H_2O_2. The reduction current increases significantly to 6.55 µA, after H_2O_2 was added to PBS. The reduction current in the CV of HRP/ZnO/ ITO bio electrode increases linearly with increase in H_2O_2 concentration from 2.5 µM to 100 µM. As the concentration of H_2O_2 increases, a greater number of electrons are generated resulting in an enhanced reduction current for the HRP/ZnO/ITO bio electrode. This may be due to the fact that ZnO acts as a better receptor of the electrons generated and transferred to the electrode via Fe^{4+}/ Fe^{3+} conversion resulting in an increased electrochemical response [**30**]. This reduction current may be due to immobilized HRP in the ZnO nanostructure, which provides a fast electron transfer reaction between the heme of HRP and the electrode surface. The heme can react with H_2O_2 to form a first intermediate of compound I, which has catalytic activity of H_2O_2 [**31**]. This catalytic mechanism is shown as below [**32**].

$$HRP(Fe^{+3}) + H_2O_2 \longrightarrow \text{Compound I } (Fe^{4+}=O) + H_2O \quad \text{---------- (1)}$$

$$\text{Compound I } (Fe^{4+}=O) + e + H^+ \longrightarrow \text{Compound II} \quad \text{---------- (2)}$$

$$\text{Compound II} + e + H^+ \longrightarrow HRP(Fe^{+3}) + H_2O \quad \text{---------- (3)}$$

The response time of the bio-electrode based on the ZnO matrix is found to be fast (about 10 sec) which may be because of the fast electron communication feature of ZnO matrix. The limit of detection was also estimated as 0.2 µM for PLD synthesized ZnO based biosensor. The Michaelis – Menten constant (K_m) which is the reflection of enzymatic affinity has

been estimated as 0.166 µM using Lineweaver- Burk equation [**33**] as given below.

$$\frac{1}{I} = \frac{1}{I_{max}} + \frac{K_m}{I_{max} C}$$

where, I is the current, I_{max} is the maximum current and C is the H_2O_2 concentration. The lower value of K_m reflects the higher activity of HRP enzyme towards its analyte (H_2O_2). This also indicates that the HRP/ ZnO/ ITO bio electrode based on ZnO nanostructure has a high affinity towards H_2O_2 which may be due to strong binding of HRP on to ZnO matrix which favors higher enzyme loading [**34**]. The ZnO nanostructure provides larger and uniform sites for immobilization of HRP and facilitates electron transfer. Sensitivity of prepared electrode for H_2O_2 is found to be 0.034 µA/ µMcm^2 [**Fig. 3(d)**].

Table 1. Performance of biosensors based on direct electron transfer of HRP in ZnO matrices.

	Linear range	Sensitivity	Detection limit	K_m	References
waxberry-like ZnO NR/GCE	1.5×10^{-4} – 15mM	---	1.15×10^{-4} mM	--	[14]
HRP/ ZnO NR/ ITO	0.5- 9 mM	7.447 µA/ mMcm^2	0.3 mM	3.426 mM	[30]
HRP/ ZnO NR/ Au wire	1.9- 2.5 µM	36.28 µA/ mM	2.2 µM	26.13 µM	[35]
Fork like ZnO NR/GCE	5×10^{-5} –7 $\times10^{-4}$ M	201.12 µA/ mM	0.3 µM	0.292 mM	[36]
HRP/ ZnO / ITO	2.5- 100 µM	0.034 µA/ µMcm^2	0.2 µM	0.166 µM	This work

The high sensitivity of HRP/ZnO/ITO bio electrode shows high electron transfer characteristics, excellent adsorption ability and effective loading of HRP on ZnO matrix. For comparison, Table I tabulates the key parameters of H_2O_2 biosensor reported in this work and previous works making use of ZnO as working electrode. From Table I, it can be seen that sensitivity, detection limit and K_m value of biosensor reported in this work is better than previous reported works. The storage ability of HRP modified ZnO/ITO bio-electrode was also investigated for 4 week period. The biosensor maintained 85 % of its primary response stored at 4 °C, indicating good stability of the ZnO based biosensor. It also shows that ZnO matrix strongly binds enzyme and prevent its activity loss so that the biosensor can maintain the HRP activity efficiently.

Conclusion

In conclusion, (002) oriented ZnO nanostructures based biosensor has been prepared for the sensing of hydrogen peroxide (H_2O_2). The ZnO nanostructures used for H_2O_2 sensing have been grown on indium tin oxide (ITO) coated glass substrate using pulse laser ablation without using any catalyst. The sensor has been prepared by immobilizing horseradish peroxidase (HRP) enzyme using physical adsorption technique on zinc oxide (ZnO) nanostructures. The HRP modified ZnO/ITO bio sensor showed a high sensitivity (0.034 µA/ µMcm^2), fast response time (~10 sec), low detection limit (0.2 µM) and good linear relationship for H_2O_2 detection. The low value of Michaelis Menten constant (0.166 µM) indicates enhanced enzyme affinity of immobilized HRP towards H_2O_2. This catalyst free pulsed laser ablated ZnO nanostructures can be exploited as efficient and potential matrix not only for bio sensing applications but also for clinical and environmental applications.

Acknowledgements

Authors would like to acknowledge TEQIP-II, Motilal Nehru National Institute of Technology (MNNIT) Allahabad and DST [SR/S2/HEP-07/2010] for providing financial assistance, and Director, MNNIT Allahabad for providing the synthesis and characterizing facilities at Center for Interdisciplinary Research Lab.

References

1. Ansari, A.; Solanki, P. R.; Malhotra, B. D. *J. Biotech*., **2009**,*142*, 179.
2. Giannoudi, L.; Piletska, E. V.; Piletsky, S. A.; Biotechnological Applications of Photosynthetic Proteins: Biochips, Biosensors and Biodevices; Springer: US, **2006**
3. Xu, J.; Shang, F.; Luong, J. H. T.; Razeeb, K. M.; Glennon, J. D. *J Biosens. Bioelectron.* **2010**, *25*, 1313.
4. Ali, M.; Tahir, M. N.; Siwy, Z.; Neumann, R.; Tremel, W.; Ensinger, W. *Anal.Chem.* **2011**, *83*, 1673.
5. Liu, X.; Luo, L.; Ding, Y.; Xu, Y.; Li, F. *J Solid state Electrochem*. **2011**, *15*, 447.
6. Ahammad, A. J. S. *J Biosens. Bioelectron.* **2013**, *9***,** 1.
7. Rahman, Md. M.; Ahammed, A. J. S.; Jin, J. H.; Ahn, S. J.; Lee, J. J. *Sensors*. **2010**, *10*, 4855.
8. Banci, L. *J. Biotech*. **1997**, *53*, 253.
9. Singh, S. P.; Arya, S. K.; Pandey, P.; Malhotra, B. D. *Appl. Phys. Lett.* **2007**, *91*, 063901.
10. Zhao, Z. L.W.; Zhang, X.; Wang, B.; Jiang, H. *Sensors.* **2010**, *10*, 1216.
11. Singh, S. P.; Arya, S. K.; Pandey, P.; Malhotra, B. D.; Saha, S.; Sreenivas, K.; Gupta, V. *Appl. Phys. Lett.* **2007**, *91*, 063901.
12. Zhiwei, Z.; Wei, L.; Xiaobing, Z.; Baoping, W.; Helong, J. *Sensors.* **2010**, *10*, 1216.
13. Shihong, C.; Ruo, Y.; Yaqin, C.; Fangxin, H. *Microchim Acta*. **2013**, *180*, 15.
14. Cao, X.; Ning, W.; Li, L. D.; Guo, L. *Sens. Actuat.* **2008**, *129*, 268.
15. Liu, J. P.; Guo, C. X.; Li, C. M.; Li, Y. Y.; Chi, Q. B.; Huang, X. T.; Liao, L.; Yu, T. *Electrochem. Commun.* **2009**, *11*, 202.
16. Yang, Y. H.; Yang, M. H.; Jlang, J. H.; Shen, G. L.; Yu, R. Q. *Chin. Chem. Lett.* **2005**, *16*, 951.
17. Duan, G. P.; Li, Y. F.; Wen, Y.; Ma, X. L.; Wang, Y.; Ji, J. H.; Wu, P.; Zhang, Z. R.; Yang, H. F. *Electroanalysis.* **2008**, *20*, 2454.
18. Wang, J.; Xu, M.; Zhao, R.; Chen, G. *Analyst*. **2010**, *135*, 1992.
19. Xiang, C.; Zou, Y.; Sun, L.X.; Xu, F. *Sensors and Actuators B.*, **2009**, *136*, 158.
20. Hussain, M.; Sun, H.; Karim, S.; Nisar, A.; Khan, M.; Haq, A.; Iqbal, M.; Ahmad, M. *J Nanopart Res.* **2016**, *18*, 95.
21. Tiwari, A.; Turner, A.P.F.; Biosensor Nanotechnology, John wiley & sons: US, **2014.**
22. Bayan, S.; Mohanta, D. *J. Appl. Phys*. **2011**, *110*, 054316.
23. Park, W. I.; Jun, Y. H.; Jung, S.W.; Yi, G. C; *Appl Phys Lett*,. **2003,** *82*, 964.

24. Yang, Y.; Tay, B. K.; Sun, X.W. *Appl. Phys. Lett.* **2007**, *91*, 071921.
25. Lin, B.; Fu, Z.; Jia, Y. G. *Appl. Phys. Lett.* **2001**, *79*, 943.
26. Gür, E.;Tüzemen, S.; Meral, K.; Onganer, Y. *Appl Phys A.* **2009**, *94***,** 549.
27. Venkatesh, P. S.; Ramakrishna, V.; Jeganathan, K. *AIP Advances*. **2013**, *3*, 082133.
28. Batra, N.; Tomar, M.; Jain, P.; Gupta, V; *J. Appl. Phys*. **2013**, *114*, 124702.
29. Yadav, S. K.; Singh, J.; Agrawal, V.; Malhotra, B. D. *Appl. Phys. Lett.* **2012**, *101*, 023703.
30. Zhang, W.; Guo, C.; Chang, Y.; Wu, F.; Ding, S. *Monatsh Chem.* **2014**, *145*, 107.
31. Lavanya, N.; Radhakrishnan, S.; Sekar, C.; *J Biosens. & Bioelectron*, **2012**, *36*, 41.
32. Zhang, Y.; Phe, Hu, N.; *Electrochimica Acta*, **2004**, *49*, 1981.
33. Xiao, Y.; Ju, H.X.; Chen, H.Y.; *Anal. Chim. Acta*, **1999**, *391*, 73.
34. Arora, K.; Tomar, M.; Gupta, V.; *J Biosens. Bioelectron.*, **2011**, *30*, 333.
35. Gu, B. X.; Xu, C.X .; Zhu, G. P.; Liu, S. Q.; Chen, L. Y.; Wang, M. L.; Zhu, J. J.; *J Phys Chem B*, **2009**, *113*, 6553.
36. Yang, Z.; Zong, X. L.; Ye, Z.; Zhao, B.; Wang, Q. L.; Wang, P.; *Biomaterials*, **2010**, *31*,7534

