Enzyme and Microbial Technology 79 (2015) 63–69

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Influence of stress in ZnO thin films on its biosensing application

Shibu Saha [a], Monika Tomar [b], Vinay Gupta [c,*]

[a] Department of Physics, Dyal Singh College, University of Delhi, Lodi Road, Delhi 110003, India
[b] Department of Physics, Miranda House, University of Delhi, Delhi 110007, India
[c] Department of Physics & Astrophysics, University of Delhi, Delhi-110007, India

ARTICLE INFO

Article history:
Received 26 April 2015
Received in revised form 24 July 2015
Accepted 24 July 2015
Available online 27 July 2015

Keywords:
ZnO
Biosensor
Enzyme immobilization
Stress

ABSTRACT

Highly c-axis oriented zinc oxide (ZnO) thin films deposited by radio frequency (RF) magnetron sputtering, under varying ambient atmosphere (oxygen and argon reactive gas mixture), were studied for biosensing application. The as-grown ZnO thin films were found to be under compressive stress. Glucose oxidase was chosen as model enzyme for studying biosensing response properties of the ZnO thin films. The present study reveals a good correlation between stress induced during thin film growth and its biosensing response characteristic. The bio-electrodes based on ZnO thin films which are under the influence of higher stress, show better sensitivity and higher enzyme loading along with a prolonged shelf life. The study highlights the importance of physical properties of thin film matrix on its biosensing application.

1. Introduction

Biosensors have seen a tremendous growth in the past decades due to their potential use in the field of health-care, self-monitoring, biological analysis, environment-monitoring and food industries. Biosensor is an analytical device that incorporates a biologically active component (receptor) in contact with an appropriate transduction element (electrical, thermal, optical, piezoelectric, etc.) for detection of the analyte bio-molecule. The matrix is that critical aspect of biosensor onto which the bio-recognition element (receptor) is immobilized. The matrix should be an electrode system with a bio-compatible microenvironment. Moreover, it should also have a good electron transfer capability. With progress in material science a number of materials such as metal oxides, self-assembled monolayers, conducting polymers and nano-composites have been used as matrix for biosensing application. The focus of the research community has been on enhancement of electron transfer rate and obtaining a good stability for immobilized biomolecules [1,2]. Hence, it is important to study the property of the matrix with respect to its biosensing response.

ZnO has found a wide range of applications [3–10]. However, the recent interdisciplinary research in the field of ZnO has led to the rapid development of a number of ZnO based biosensors [9–12]. The high electron communication feature of ZnO and its bio-compatibility has further boosted the application of ZnO as a matrix for biosensors [13]. Being a piezoelectric material, ZnO finds application in SAW [14], FBAR [15] and QCM [16] based biosensors. ZnO based hydrogen peroxide biosensor has been reported which is modulated by applying external strain [17]. On the other hand, synthesis of a rich array of ZnO nanostructures has enhanced the interest of the research community toward its application as a matrix in electrochemical biosensors [18]. However, the study on the co-relation of the physical properties of the matrix with its biosensing response is hardly available in the literature. Stress is one of the important properties of thin film that directly affects its vital behavior like conductivity and surface morphology [17,19,20]. As the unit cells undergoes compression or elongation along a crystal axis, the film is said to experience a stress along its surface. Generally stress originates in thin film during its growth or processing. Hence, the optimization of stress introduced during film growth becomes important to achieve the desirable properties appropriate for the biosensing application. Though, the induced stress may be manipulated using post deposition processing [21] but it adds to cost and turnaround time, which are the major factors to be considered in mass scale production.

The physical property and hence, the biosensing characteristic is known to be affected by external as well as intrinsic stress [17,19]. However, the external stress plays a role in case of piezoelectric biosensor [17]. For the electrochemical biosensor the intrinsic stress plays a major role as it affects the charge transfer properties of ZnO thin film [19]. It is well known, that induced stress and physical properties of the films is governed by growth kinetics which in turn strongly depends on the processing conditions [19,20]. In

* Corresponding author.
E-mail address: vgupta@physics.du.ac.in (V. Gupta).

http://dx.doi.org/10.1016/j.enzmictec.2015.07.008
0141-0229/

a previous study [19] it was observed that changing the ambient pressure affects the biosensing response characteristic which was in turn related to the deposition induced stress. The increase in stress was seen to improve the biosensing characteristic of the ZnO based biosensor [19]. However, biosensing characteristics are well known to be dependent on the surface property of the matrices which are also affected by the variation in ambient pressure [20]. Hence, the variation in the biosensing characteristic may also be attributed to surface properties instead of induced stress. If a similar dependence may be observed in the biosensing characteristic by variation in stress induced by other parameters then the ambiguity may be resolved. Variation in the processing gas composition in the deposition ambient is also known to affect the growth induced stress in thin films [20].

In the present work, ZnO thin films have been grown by RF-sputtering under a reactive growth environment of oxygen and argon. The variation of stress in the thin film matrix, required in the study, was obtained by changing the processing ambient (argon to oxygen ratio) keeping the deposition pressure fixed. For studying the biosensing response of the ZnO thin film based matrices (grown under different ambient gas mixture) glucose oxidase (GO*x*) has been chosen as the model enzyme because glucose biosensors are one of the most heavily investigated biosensors. GO*x* specifically catalyses the oxidation of β-D-glucose to gluconic acid. The reaction is characterized by concurrent release of hydrogen peroxide [22]. The enzyme (GO*x*) is immobilized on the thin film matrix (ZnO) for specific detection of glucose. The relationship observed between the biosensing characteristics and the stress induced during growth in the ZnO thin films is presented.

2. Experiment

In the present study, ZnO thin films were deposited on indium tin oxide (ITO) coated 1737 corning glass substrate (conducting bottom electrode) by RF-magnetron sputtering technique under imbalanced mode for biosensing response studies. The thin films were deposited using zinc (Zn) metal target (99.99% pure) under typical deposition conditions given in Table 1 and varying the argon to oxygen ratio in the sputtering gas mixture. The oxygen percentage was varied from 50% to 100% in the reactive gas mixture of argon and oxygen. It is important to point out that since metal Zn is used as the target and the formation of ZnO film is based on reactive sputtering in oxygen environment, a low oxygen concentration hampers formation of the metal oxide. Though the films, grown in an ambient having lesser oxygen than argon, have higher conductivity but they are metallic in nature. The films grown in an ambient with more argon are blackish in color and does not support enzyme loading. Hence, biosensing studies have not been carried out on films grown in an ambient having oxygen concentration less than 50%. ZnO thin films of constant thickness (80 nm) were grown by optimizing the deposition time according to the rate of growth under different ambient conditions. The thickness of the film was measured using a surface profiler (Dektek 150). The structural property of the film was studied using a Bruker X-ray diffractometer (XRD). Optical transmission studies and the photometric assay were carried out using Perkin Elmer (Lambda 35) UV-visible spectrometer. The dc-conductivity of the ZnO thin films was measured using a semiconductor characterization setup (Keithley 236 source measure unit). Metal-insulator-metal (MIM) structure with platinum (Pt) as the top and bottom electrodes was used for electrical studies as has been discussed elsewhere [19]. The cyclic voltammetry is the technique being used by the research community for bio-electroanalysis of the electrochemical biosensors [9,13,19,23]. Cyclic voltammetry (Gamry potentiostat/galvanostat Reference 300 model) was employed for electrochemical and biosensing response studies. The cyclic voltammograms were obtained in phosphate buffer saline (PBS) solution, containing 5 mM $[Fe(CN)_6]^{3-/4-}$, using the three electrode configuration with Ag/AgCl as the reference electrode. In the three electrode system, ZnO thin films deposited on ITO coated glass (ZnO/ITO/Glass) were made the working electrode while a platinum foil was used as the counter electrode. Glucose oxidase (GO*x*) is immobilized on the ZnO/ITO/Glass by the physical adsorption technique to form the GO*x*/ZnO/ITO/Glass bio-electrode. The details of enzyme immobilization and formation of the bio-electrode have been discussed elsewhere [19].

Table 1
Processing parameters for the deposition of ZnO thin films.

Target	Zinc (6″ diameter; 99% pure)
Substrate	ITO coated 1737 corning glass (for biosensing)
RF power	300 W
Sputtering pressure	50 mTorr
Sputtering gas composition	50–100% O_2
Substrate temperature	Room temperature
T–S distance	8.5 cm

3. Results

3.1. X-ray diffraction study

Fig. 1 shows the X-ray diffraction (XRD) patterns of the ZnO thin films deposited on corning glass substrate under the conditions given in Table 1, with a varying oxygen percentage (50–100%) in the reactive gas mixture (Ar–O_2). It is important to point out that the thickness of all the films were kept constant at 80 nm. The XRD data of all the deposited films show a preferred orientation of the crystallites along the (0 0 2) plane of the ZnO wurtzite structure. The results indicate the growth of highly c-axis oriented thin films, where c-axis is normal to the substrate plane. The tendency of ZnO thin films to grow with c-axis orientation is due to the minimum surface energy of the (0 0 2) plane. Though, it is surprising to have a preferred (0 0 2) orientation in the 80 nm ZnO thin film, but the possible reason could be the quick nucleation toward the (0 0 2) plane, having lowest surface energy, due to the bombardment of substrate surface by high energy sputtered species created in the unbalanced magnetron sputtering.

The Bragg's angle (2θ) for (0 0 2) XRD peak of the ZnO thin films is observed to vary from 34.16° to 34.26° with increase in oxygen

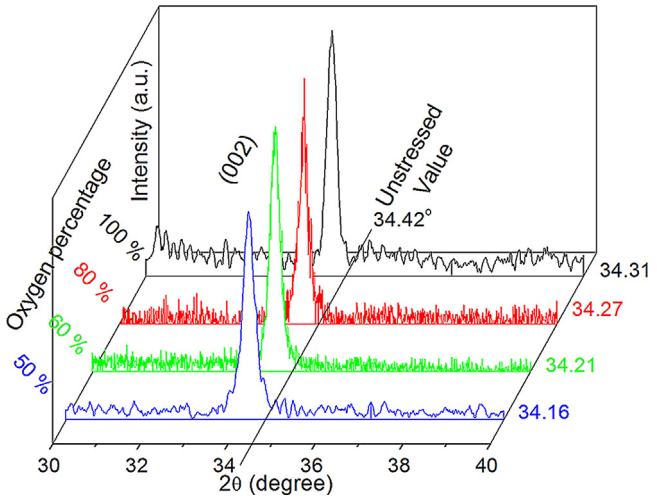

Fig. 1. XRD spectra of ZnO films deposited under different ambient oxygen concentrations in the sputtering gas mixture (Ar–O_2).

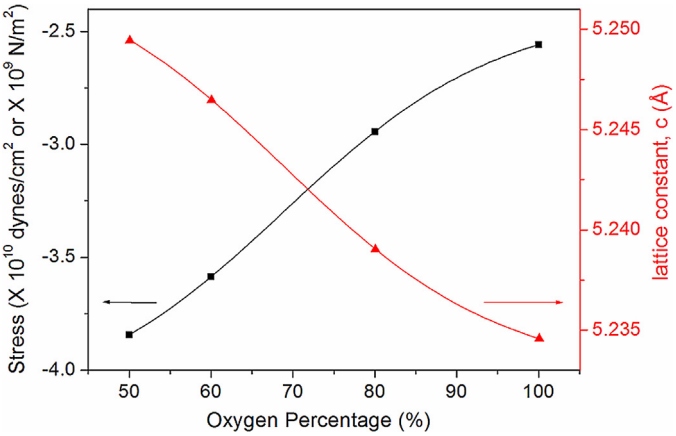

Fig. 2. Variation in compressive stress and lattice constant (c) of ZnO film deposited under varying oxygen concentration in the sputtering gas mixture ($Ar–O_2$).

Table 2
XRD related parameters for ZnO films grown under various deposition conditions.

Oxygen percentage in deposition ambient (%)	2θ (°)	c (Å)	Stress ($\times 10^{10}$ dynes/cm^2)
50	34.16	5.249	−3.84
60	34.18	5.246	−3.58
80	34.23	5.239	−2.94
100	34.26	5.235	−2.56

percentage from 50% to 100% in the growth ambient ($Ar–O_2$ mixture). All the XRD peaks are obtained at a 2θ value, which is less than the corresponding value reported for bulk ZnO ($2\theta = 34.42°$) [20]. The shift of the 2θ toward lower angle is due to the development of a compressive stress in the ZnO films during the growth process [20]. The stress decreases from from -3.84×10^{10} dynes/cm^2 to -2.56×10^{10} dynes/cm^2 with increase in oxygen percentage from 50% to 100%. The variation of stress and lattice constant '*c*' (estimated from the XRD data) for the ZnO thin films deposited under varying oxygen percentage is shown in Fig. 2. It may be clearly seen that the compressive stress in the thin film decreases with increasing oxygen percentage which leads to reduction in the ZnO unit cell along c-axis. The various parameters estimated from the XRD data are summarized in Table 2.

In the ZnO thin films grown under higher oxygen percentage, the probability of introduction of Zn interstitials is reduced while point defects related to oxygen interstitials and oxygen antisites may appear. Oxygen being smaller in size than zinc contributes in decreasing the size of the unit cell along the c-axis and hence, a decrease in crystallite size of ZnO is observed with increasing the oxygen partial pressure in the sputtering gas mixture (Table 2). The oxygen interstitial or oxygen antisite defects in ZnO act as acceptor impurities and are expected to capture free electrons from the bulk of ZnO. Therefore, the electron communication capability is expected to reduce for the ZnO matrix deposited under oxygen rich conditions.

3.2. Current–voltage characteristic

The current–voltage (IV) characteristics of the ZnO thin films have been studied in MIM configuration and were found to be of ohmic nature. The dc-conductivity (σ_{dc}) of the ZnO thin films grown under different oxygen percentages were measured at a fixed bias of 1.0 V and is depicted in Fig. 3. The room temperature σ_{dc} of the ZnO thin films decreased from $3.7 \times 10^{-7}\ \Omega^{-1}\ cm^{-1}$ to $3.1 \times 10^{-7}\ \Omega^{-1}\ cm^{-1}$ with increase in the oxygen percentage from 50% to 100% in the processing gas mixture. The decrease in the dc-

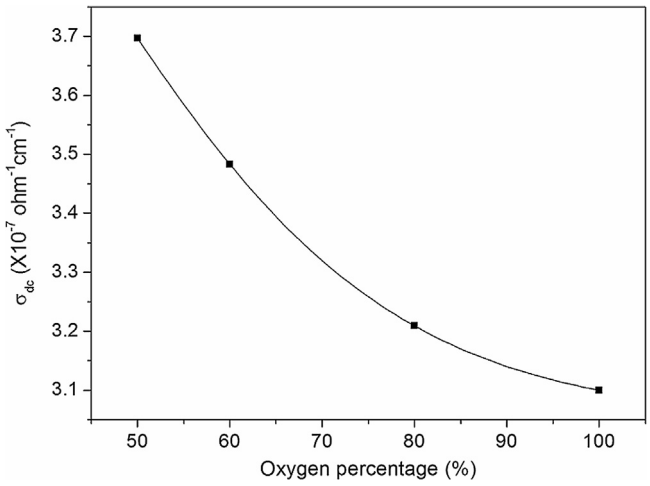

Fig. 3. Variation in dc-conductivity of ZnO thin film deposited in varying oxygen concentrations in sputtering gas mixture ($Ar–O_2$).

conductivity of the ZnO film with increasing oxygen content in the processing gas indicates the reduction in its electron communication capability. This is attributed to the presence of point defects related to oxygen at the interstitial or Zn antisite which acts as trap centers for the free electrons and hence, reduces the charge carriers taking part in conduction.

The dc-conductivity of the ZnO thin films, deposited under different oxygen concentrations, is recorded at higher temperatures. The σ_{dc} of the ZnO thin films is shown in Fig. 4 as a function of reciprocal temperature (1000/*T*). At higher temperatures the dc-conductivity shows an increasing trend. The variation of σ_{dc} with temperature is arrhenious in nature and is given by the relation;

$$\sigma_{dc} = \sigma_o \exp(-\Delta E/kT)$$

where, ΔE is the activation energy, T is the temperature, σ_o is the pre-exponential factor, and k is the Boltzmann's constant. The $1000/T$ vs. $\ln \sigma_{dc}$ graph may be used to obtain the activation energy for electrical conduction of the ZnO thin films grown at various oxygen percentages in the deposition atmosphere. The activation energy was found to be in the range 0.09–0.13 eV for all the samples, and is observed to increase with increase in the ambient oxygen concentration (inset of figure 4). The increased activation energy suggests an enhancement in the role of trap centers in the conduction mechanism in the ZnO films grown at higher oxygen

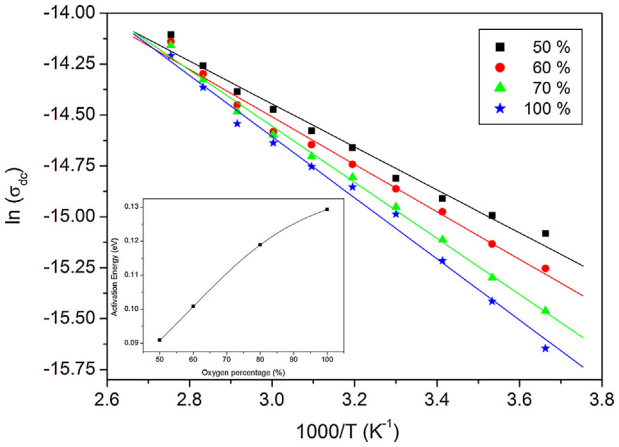

Fig. 4. ln σ_{dc} vs. 1000/T graph of ZnO films deposited under different ambient oxygen concentrations sputtering gas mixture ($Ar–O_2$) (Inset shows variation of activation energy with oxygen concentration).

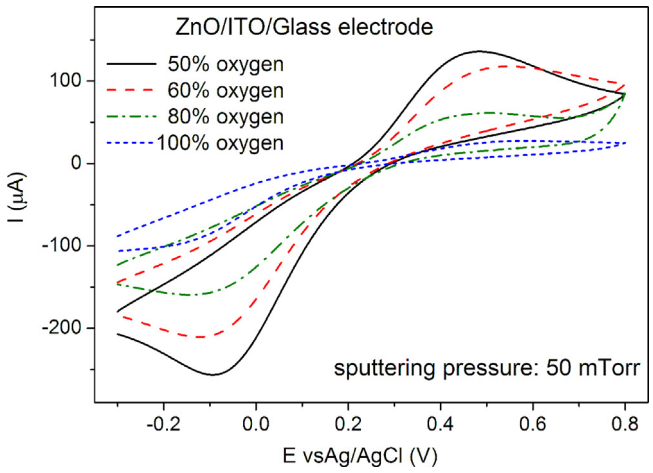

Fig. 5. CV spectra of ZnO films deposited under various oxygen percentage in sputtering gas mixture (in PBS solution, containing 5 mM $[Fe(CN)_6]^{3-/4-}$, with Ag/AgCl as the reference electrode).

concentration in the deposition ambient. Thus, it may be inferred that the films grown at a lower oxygen concentration (50%) should exhibit a higher electron transfer capability.

3.3. Cyclic voltammetry studies

The cyclic voltammograms (CV) of the ZnO/ITO/Glass electrodes deposited under varying oxygen percentage in the sputtering ambient are shown in Fig. 5. The cyclic voltammograms are seen to be strongly dependent on the processing condition of the ZnO thin film. The peak oxidation current is observed to decrease significantly for the electrodes having ZnO films grown under higher oxygen percentage in the sputtering gas mixture (Fig. 5). The variation of peak oxidation current with oxygen concentration in the growth ambient is shown in Fig. 6. The peak oxidation current is maximum (135.8 μA) for ZnO/ITO/Glass electrode having ZnO thin film deposited under 50% oxygen content in the sputtering gas mixture. Surface concentration of redox species (Γ) may be computed using the Brown-Anson model given by;

$$I_p = \frac{n^2F^2\Gamma A\nu}{4RT}$$

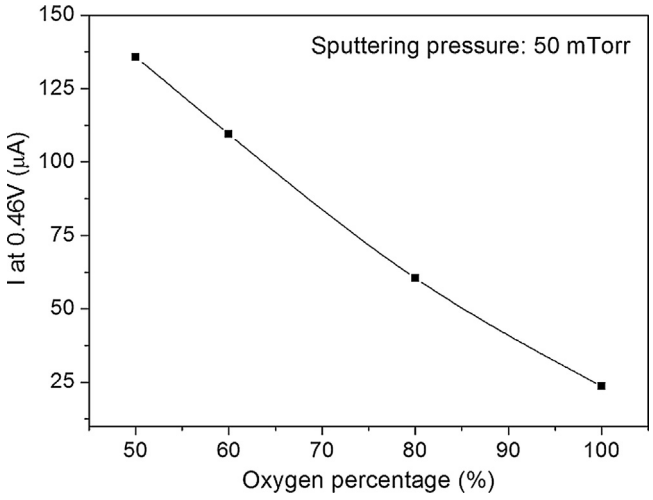

Fig. 6. Variation in the peak oxidation current of the ZnO films deposited under various oxygen percentages.

Table 3
Values of surface concentration of redox species (Γ) for ZnO/ITO/Glass electrodes having ZnO matrix grown under varying oxygen percentage in the sputtering gas mixture.

Oxygen percentage (%)	Γ (nano moles/cm^2)
50	3.36
60	2.62
80	1.44
100	0.71

where, I_p is the peak current obtained at a scan rate of ν, F is the Faraday's constant (96,485 Cmol^{-1}), R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (room temperature = 300 K), A is the area of the prepared electrode (0.5 cm^2) and n is the number of electrons taking part in the redox reaction which is '1' in our case, since only one electron is involved in the oxidation/reduction of $[Fe(CN)_6]^{3-/4-}$ ions [1,24]. The Γ computed for the electrodes based on ZnO films grown under different ambient are tabulated in Table 3. It may be noted that the surface concentration of redox species decreases for ZnO thin films grown in an ambient having higher oxygen concentrations. The observations related to the CV response are attributed to the decrease in the dc-conductivity due to incorporation of acceptor type defects in the ZnO thin film grown under richer oxygen ambient.Biosensing responses of the bio-electrodes (GO*x*/ZnO/ITO/Glass) having ZnO thin films deposited under various oxygen percentages (50–100%) were studied. The CVs of the GO*x*/ZnO/ITO/Glass bio-electrodes show a diminished redox current (red colored dotted CVs in Fig. 7) as compared to that obtained for the ZnO/ITO/Glass electrodes (solid black colored CVs in Fig. 7) due to immobilization of enzyme, on the ZnO matrix, which is a macro-molecule of non-conducting nature. Since a well defined redox peak could not be observed for the GO*x*/ZnO/ITO/Glass bio-electrodes having ZnO thin film deposited under 80% and 100% oxygen gas concentration in the sputtering gas mixture (Ar–O_2), therefore, the glucose sensing response was studied for the bio-electrodes having ZnO thin film grown in an ambient having 50% and 60% oxygen. CV spectra of the GO*x*/ZnO/ITO/Glass bio-electrodes (with ZnO grown in 50% and 60% oxygen concentrations) obtained on addition of different concentrations of glucose (0 mM to 22.22 mM or 0 mg/dl to 400 mg/dl) in the electrolyte solution are shown in Fig. 8((a): 50% oxygen concentration and (b): 60% oxygen concentrations). The redox current is observed to increase continuously with successive addition of glucose in the electrolytic solution (Fig. 8). The proportional increase of the redox current is due to enrichment of electrons, in the electrolyte solution, during the catalytic oxidation of glucose in presence of the immobilized enzyme GO*x*. Fig. 9 shows the variation of the peak oxidation current as a function of glucose concentrations for the bio-electrodes based on ZnO thin films grown in an ambient (a mixture of Ar–O_2) having 50% and 60% oxygen. It may be seen that both the bio-electrodes show a linear increase in the peak oxidation current with an increase in the glucose concentration up to 22.22 mM and saturates thereafter. The sensitivity of the bio-electrode (rate of increase of current with glucose concentration as calculated from the calibration curve) is observed to be much more for the bio-electrode based on ZnO matrix deposited under an ambient having 50% oxygen in the gas mixture (1.27 μA mM^{-1} cm^{-2}) as compared to the one grown under 60% oxygen concentration (0.47 μAmM^{-1} cm^{-2}). Moreover, it is interesting to note that the lower detection limit of the bio-electrodes is also found to deteriorate for matrix grown in an oxygen rich ambient (Table 4).

The Michaelis–Menten constant (K_m^{app}) quantifies the affinity of the enzyme (GO*x* in our case) toward its bio-analyte (glucose) and may be determined using Hanes plot [19,25]. The K_m^{app} value for the GO*x* immobilized on ZnO based matrix deposited in an ambient having 50% oxygen is found to be lower than that grown in

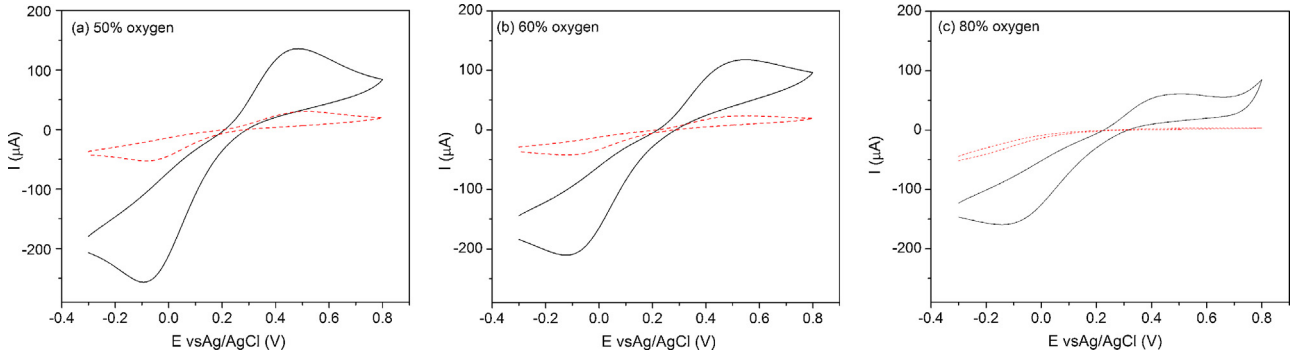

Fig. 7. CV of ZnO/ITO/Glass electrode (black) and GO*x*/ZnO/ITO/Glass bio-electrode (red) for ZnO thin films grown in an ambient, having (a) 50% (b) 60% and (c) 80% oxygen.

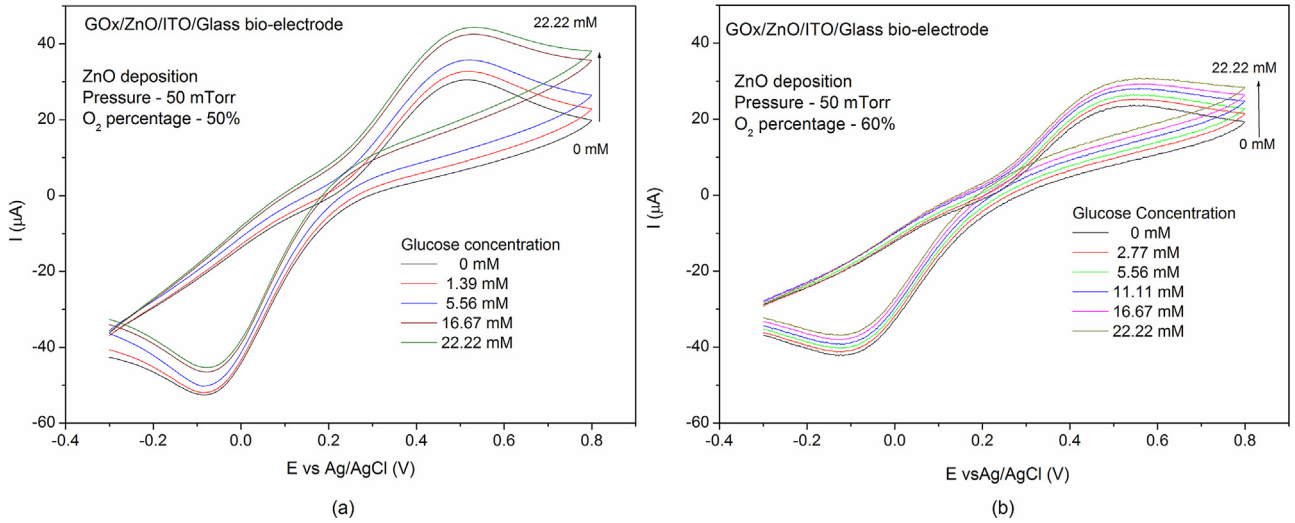

Fig. 8. Variation in the CV of GOx/ZnO/ITO/Glass bio-electrode with changing glucose concentration based on ZnO thin films grown in an ambient, having (a) 50% and (b) 60% oxygen.

60% oxygen (Table 4). The typical biosensing response parameters determined for the bio-electrodes are listed in Table 4. It is important to note that the estimated values of K_m^{app} for both the bio-electrodes are lower than the corresponding value for free GO*x* (~27 mM [19]), which implies increased affinity of the immobilized GO*x* enzyme toward glucose. The ZnO matrix provides a suitable microenvironment that assists effective immobilization of enzyme on the surface of the matrix and an enhanced electron communication between the enzyme's active site and the electrode.

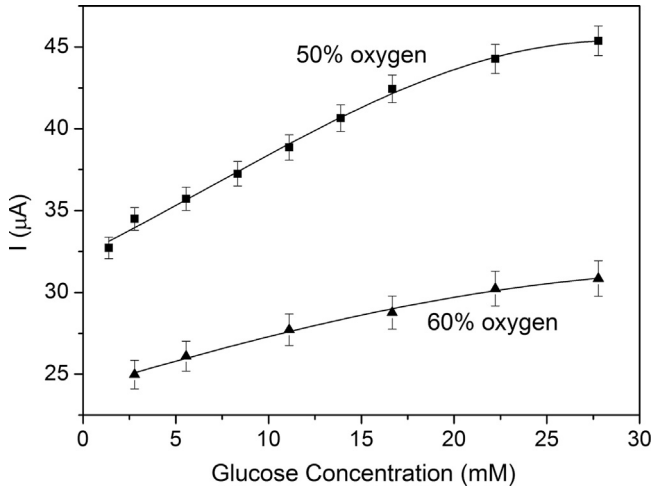

Fig. 9. Linearity curves of the bio-electrodes having ZnO thin film matrix grown under different ambient oxygen concentration.

3.4. Photometric assay

The biosensing response of a bio-electrode may also be studied using the photometric assay. In the process, sensing is based on change in the absorbance at a particular wavelength in contrast to the change in current recorded in electrochemical sensing [19]. The details of photometric enzyme assay have been given in our previous report [19]. The change in absorbance at a fixed wavelength (500 nm) recorded as a function of glucose concentration is shown in Fig. 10. The apparent enzyme activity (amount of enzyme bound on the surface of the matrix) has been calculated as described in reference [19]. The value of apparent enzyme activity (α_{app}^{enz}) is found to increase from 0.98×10^{-2} to 1.18×10^{-2} Ucm^{-2} for the GO*x*/ZnO/ITO/Glass bio-electrode based on ZnO thin films deposited with decreasing oxygen content from 100% to 50% in the processing gas mixture (Table 4). The better enzyme loading obtained for the GO*x*/ZnO/ITO/Glass bio-electrode having ZnO thin film deposited under 50% oxygen in the sputtering gas mixture, may be attributed to the rough surface morphology formed due to the high stress induced in the ZnO thin film during its growth.

It is interesting to note that the bio-electrode based on ZnO thin film deposited in oxygen deficient atmosphere (50% O_2 in sputtering gas mixture) possess a higher shelf-life as compared to the ones deposited in oxygen rich conditions (Table 4). The rough microstructure of the stressed thin film is expected to provide a suitable

Table 4
Critical biosensing parameters of GOx/ZnO/ITO/Glass bio-electrode toward glucose having ZnO matrix grown under varying O_2 concentration in the sputtering gas mixture.

Oxygen percentage (%)	Sensitivity ($\mu AmM^{-1}cm^{-2}$)	Lower detection limit (mM)	Km (mM)	a_{enz} (X 10^{-2} U cm^{-2})	Shelf life (weeks)
50	1.27	0.05	1.11	1.18	11
60	0.47	0.07	1.28	1.09	9
80	–	–	–	1.04	8
100	–	–	–	0.98	8

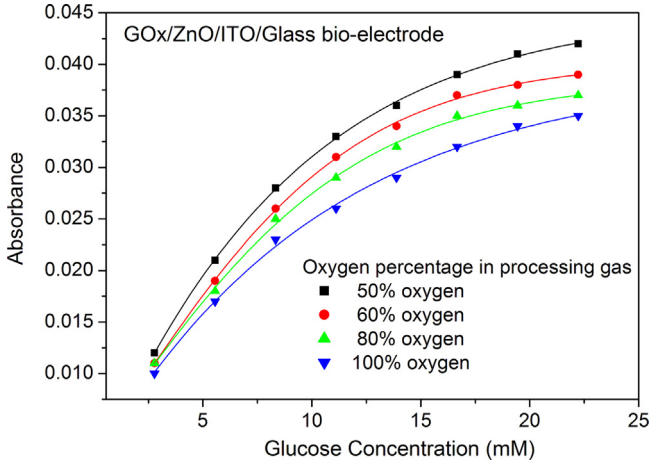

Fig. 10. Photometric assay of the bio-electrodes having ZnO grown under different ambient oxygen concentrations.

micro-environment for higher enzyme loading and thus, retaining the enzyme activity.

4. Discussion

The biosensing response of the bio-electrodes is seen to be strongly influenced by the intrinsic stress induced in the ZnO thin film grown under different oxygen concentrations. An oxygen rich deposition environment induces more oxygen in the lattice as point and antisite defects. These defects directly affect the unit cell of ZnO by shortening it along the c-axis (Table 2) and in the process relieving the stress in the film. Thus the films grown in an oxygen deficient condition are more stressed than those grown in an oxygen rich ambient. It is important to point out here that the oxygen defects (vacancies and antisites) create acceptor levels in the forbidden region and hence, act as trap centers for the charge carriers. Thus, the films under a higher stress are expected to exhibit an enhanced charge communication feature as compared to those under a comparatively lower stress. The biosensing application is highly dependent on the electron transfer capability of the matrix. The cyclic voltammetry study shows that the redox current is more in the ZnO thin film which is under the influence of a higher stress. Moreover, it is observed that the films under high stress show a better sensitivity and enhanced response. These observations are supported by the conductivity measurements also. The dc-conductivity study is a direct signature of the charge transfer capacity of the matrix. It is observed from the IV studies that the σ_{dc} decreases with decrease in the stress induced in the ZnO thin films during their growth. This has been attributed to the oxygen related defects introduced during the growth in an oxygen rich ambient. It is further interesting to note that the activation energy, computed from the temperature dependent conductivity measurements, also increases with relieving of stress in the ZnO thin films and directly supports the CV and IV observations. It is well known that the *n*-type semiconducting nature of ZnO is due to the shallow donor levels created by the zinc interstitials and oxygen vacancies [26]. However, the donor levels which assist in *n*-type conduction are competed by the acceptor levels created by the oxygen rich impurities like the vacancies and antisites [26]. Thus, the lower activation energy in the ZnO thin films, grown in scarce oxygen ambient, is a manifestation of deficiency of oxygen related acceptor impurities. The lower activation energy and high dc-conductivity obtained in the ZnO thin films under high intrinsic stress are clearly related to the decrease in acceptor type of defects which has been observed in ZnO thin films grown in an atmosphere having lesser oxygen concentration. The lower activation energy indicates that the films provide a better path for electron transfer from the redox center to the electrode. Moreover, the ZnO thin films under the influence of a higher stress provide a rough microstructure which provides a higher surface area for enzyme loading and proves to be more suitable for retaining their bio-activity [13]. Thus, the ZnO thin films under the influence of higher stress (induced during its growth) exhibit an enhanced biosensing response.

5. Conclusions

The effect of intrinsic stress in ZnO thin films on their biosensing application has been studied. The biosensing response characteristic is seen to be strongly associated to the intrinsic stress of the matrix, which is directly related with the physical properties of the thin film. It is observed that the films with high intrinsic stress prove to be more suitable for biosensing application as it provides an enhanced electron communication characteristic. Thus, the physical properties of the matrix strongly impact the biosensing response. Hence, the properties of the thin film matrix are needed to be well understood to achieve an optimum characteristic of the corresponding bio-electrode.

Acknowledgements

Authors are thankful to University Grants Commission (UGC) and Department of Science and Technology (DST) for financial support.

References

[1] R. Khan, A. Kaushik, P.R. Solanki, A.A. Ansari, M.K. Pandey, B.D. Malhotra, Zinc oxide nanoparticles-chitosan composite film for cholesterol biosensor, Anal. Chim. Acta 616 (2008) 207–213.
[2] S. Saha, S.K. Arya, S.P. Singh, K. Sreenivas, B.D. Malhotra, Vinay Gupta, Zinc oxide–potassium ferricyanide composite thin film matrix for biosensing applications, Anal. Chim. Acta 653 (2008) 212–216.
[3] H. Liu, Z. Wu, J. Hu, Q. Song, B. Wu, H.L. Tam, Q. Yang, W.H. Choi, F. Zhu, Efficient and ultraviolet durable inverted organic solar cells based on an aluminum-doped zinc oxide transparent cathode, Appl. Phys. Lett. 103 (2013) 043309.
[4] L. Museur, J.-P. Michel, P. Portes, A. Englezis, A. Stassinopoulos, D. Anglos, A.V. Kanaev, Femtosecond UV laser non-ablative surface structuring of ZnO crystal: impact on exciton photoluminescence, J. Opt. Soc. Am. B 27 (2010) 531–535.
[5] L.C. Campos, M.H.D. Guimarães, A.M.B. Goncalves, S. de Oliveira, R.G. Lacerda, ZnO UV photodetector with controllable quality factor and photosensitivity, AIP Adv. 3 (2013) 022104.
[6] Y.H. Ko, S.H. Lee, J.S. Yu, Performance enhanced piezoelectric ZnO nanogenerators with highly rough Au electrode surfaces on ZnO submicrorod arrays, Appl. Phys. Lett. 103 (2013) 022911.
[7] J. Pan, S. Wang, D. Zhang, J. Hu, Q. Chen, J. Wang, Magnetism and stability of noncompensated anion-cation codoped ZnO, J. Appl. Phys. 113 (2013) 043915.

[8] S.C. Hung, W.Y. Woon, S.M. Lan, F. Ren, S.J. Pearton, Characteristics of carbon monoxide sensors made by polar and nonpolar zinc oxide nanowires gated AlGaN/GaN high electron mobility transistor, Appl. Phys. Lett. 103 (2013) 083506.
[9] N. Batra, M. Tomar, P. Jain, V. Gupta, Laser ablated ZnO thin film for amperometric detection of urea, J. Appl. Phys 114 (2013) 124702.
[10] S.K. Arya, S. Saha, J.E.R.- Vick, V. Gupta, S. Bhansali, S.P. Singh, Recent advances in ZnO nanostructures and thin films for biosensor applications: review, Anal. Chim. Acta 737 (2012) 1–21.
[11] L. Zhang, Y. Wang, J. Wang, J. Shi, K. Deng, W. Fu, rhEPO/EPO discrimination with ultrasensitive electrochemical biosensor based on sandwich-type nano-Au/ZnO sol–gel/nano-Au signal amplification, Biosens. Bioelectron. 50 (2013) 217–223.
[12] A. Cai, A. Guo, Y. Chang, Y. Sun, S. Xing, Z. Ma, Fast synthesis of DNA-assisted flower-like ZnO superstructures with improved photocatalytic and antibacterial performances, Mater. Lett. 111 (2013) 158–160.
[13] S.P. Singh, S.K. Arya, P. Pandey, B.D. Malhotra, S. Saha, K. Sreenivas, V. Gupta, Cholesterol biosensor based on rf sputtered zinc oxide nanoporous thin film, Appl. Phys. Lett. 91 (2007) 063901.
[14] S. Krishnamoorthy, A.A. Iliadis, T. Bei, G.P. Chrousos, An interleukin-6 $ZnO/SiO_2/Si$ surface acoustic wave biosensor, Biosens. Bioelectron. 24 (2008) 313–318.
[15] Z. Yan, X.Y. Zhou, G.K.H. Pang, T. Zhang, W.L. Liu, J.G. Cheng, Z.T. Song, S.L. Feng, L.H. Lai, J.Z. Chen, Y. Wang, ZnO-based film bulk acoustic resonator for high sensitivity biosensor applications, Appl. Phys. Lett. 90 (2007) 143503.
[16] R. Hao, H. Song, G. Zuo, R. Yang, H. Wei, D. Wang, Z. Cui, Z. Zhang, Z. Cheng, X. Zhang, DNA probe functionalized QCM biosensor based on gold nanoparticle amplification for *Bacillus anthracis* detection, Biosens. Bioelectron. 26 (2011) 3398–3404.
[17] Y. Han, C. Gao, H. Zhu, S. Chen, Q. Jiang, T. Li, M. Willander, X. Cao, N. Wang, Piezotronic effect enhanced nanowire sensing of H_2O_2 released by cells, Nano Energy 13 (2015) 405–413.
[18] S. Xu, Z.L. Wang, One-dimensional ZnO nanostructures: solution growth and functional properties, Nano Res. 4 (2011) 1013–1098.
[19] S. Saha, V. Gupta, Influence of surface defects in ZnO thin films on its biosensing response characteristic, J. Appl. Phys. 110 (2011) 064904.
[20] R. Menon, K. Sreenivas, V. Gupta, Influence of stress on the structural and dielectric properties of rf magnetron sputtered zinc oxide thin film, J. Appl. Phys. 103 (2008) 094903.
[21] V. Gupta, A. Mansingh, Influence of postdeposition annealing on the structural and optical properties of sputtered zinc oxide film, J. Appl. Phys. 80 (1996) 1063.
[22] L.C. Clark, C. Lyons, Electrode systems for continuous monitoring in cardiovascular surgery, Ann. N. Y. Acad. Sci. 102 (1962) 29–45.
[23] F. Zhang, X. Wang, S. Ai, Z. Sun, Q. Wan, Z. Zhu, Y. Xian, L. Jin, K. Yamamoto, Immobilization of uricase on ZnO nanorods for a reagentless uric acid biosensor, Anal. Chim. Acta 519 (2004) 155–160.
[24] S.J.R. Prabhakar, S.S. Narayanan, Amperometric determination of paracetomol by a surface modified cobalt hexacyanoferrate graphite wax composite electrode, Talanta 72 (2007) 1818–1827.
[25] E. Rogers, Handbook of Biosensors & Electronic Noses Medicine, Food, and the Environment, CRC, Press, USA, 1997.
[26] A. Janotti, C.G. Van-de-Walle, Fundamentals of zinc oxide as a semiconductor, Rep. Prog. Phys. 72 (2009) 126501.