Enzyme and Microbial Technology 96 (2017) 67–74

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Mediator-free interaction of glucose oxidase, as model enzyme for immobilization, with Al-doped and undoped ZnO thin films laser-deposited on polycarbonate supports

Fidal V.T.K.P.[a], Saikumar Inguva[b], Satheesh Krishnamurthy[b,1], Enrico Marsili[c,2], Jean-Paul Mosnier[b], Chandra T.S.[a,*]

[a] Bhupat and Jyoti Mehta School of Biosciences, Department of Biotechnology, Indian Institute of Technology, Madras, Chennai-600036, India
[b] School of Physical Sciences and National Centre for Plasma Science and Technology, Dublin City University, Collins Avenue, Dublin9, Ireland
[c] School of Biotechnology, Dublin City University, Collins Avenue, Dublin9, Ireland

ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form
19 September 2016
Accepted 21 September 2016
Available online 23 September 2016

Keywords:
Glucose oxidase
ZnO
Al-doped ZnO
Polycarbonate

ABSTRACT

Al doped and undoped ZnO thin films were deposited by pulsed-laser deposition on polycarbonate sheets. The films were characterized by optical transmission, Hall effect measurement, XRD and SEM. Optical transmission and surface reflectometry studies showed good transparency with thicknesses ~100 nm and surface roughness of 10 nm. Hall effect measurements showed that the sheet carrier concentration was -1.44×10^{15} cm^{-2} for AZO and -6×10^{14} cm^{-2} for ZnO. The films were then modified by drop-casting glucose oxidase (GOx) without the use of any mediators. Higher protein concentration was observed on ZnO as compared to AZO with higher specific activity for ZnO (0.042 U mg^{-1}) compared to AZO (0.032 U mg^{-1}), and was in agreement with cyclic voltemmetry (CV). X-ray photoelectron spectroscopy (XPS) suggested that the protein was bound by dipole interactions between AZO lattice oxygen and the amino group of the enzyme. Chronoamperometry showed sensitivity of 5.5 μA mM^{-1} cm^{-2} towards glucose for GOx/AZO and 2.2 μA mM^{-1} cm^{-2} for GOx/ZnO. The limit of detection (LoD) was 167 μM of glucose for GOx/AZO, as compared to 360 μM for GOx/ZnO. The linearity was 0.28–28 mM for GOx/AZO whereas it was 0.6–28 mM for GOx/ZnO with a response time of 10s. Possibly due to higher enzyme loading, the decrease of impedance in presence of glucose was larger for GOx/ZnO as compared to GOx/AZO in electrochemical impedance spectroscopy (EIS). Analyses with clinical blood serum samples showed that the systems had good reproducibility and accuracy. The characteristics of novel ZnO and AZO thin films with GOx as a model enzyme, should prove useful for the future fabrication of inexpensive, highly sensitive, disposable electrochemical biosensors for high throughput diagnostics.

1. Introduction

ZnO is a well-established material for bio-sensing applications due to its unique features such as high catalytic efficiency, biocompatibility, strong adsorbance, electrochemical activity, easy availability and long term stability [1–3]. It has other advantages over other metal oxide thin films, including the possibility of low temperature deposition, facile fabrication of nanostructures and attractive optoelectronic and piezoelectric properties [4,5].

Doping is a common method to alter the electronic and optical properties of ZnO thin films [6]. Aluminum (Al) and gallium (Ga) are n-type dopants that increase the concentration of free electrons, thereby improving the conductivity of ZnO films [7,8]. Al-doping of ZnO (AZO) had shown higher reactivity and optical transmittance than ZnO and is preferred dopant for the fabrication of transducer devices [8,9]. The resistivity is dependent not only on the Al concentration but also on the oxygen partial pressure used during the plasma assisted deposition [10]. Aragones et al. reported that doping ZnO with increasing concentrations of Al (2–11 at.%) increased the carrier concentration from 10^{19} to 7×10^{20} cm^{-3} [11]. Al doped ZnO is more sensitive as compared to undoped ZnO towards adsorbed species on the surface. Therefore

* Corresponding author.
E-mail address: chandrasainathan@gmail.com (C. T.S.).
[1] Present address: Department of Engineering and Innovation Materials Engineering, Open University, MK7 6AA, UK.
[2] Present address: Singapore Centre for Life Sciences Engineering, Nanyang Technological University, 60 Nanyang drive, 637551, Singapore.

http://dx.doi.org/10.1016/j.enzmictec.2016.09.012
0141-0229/

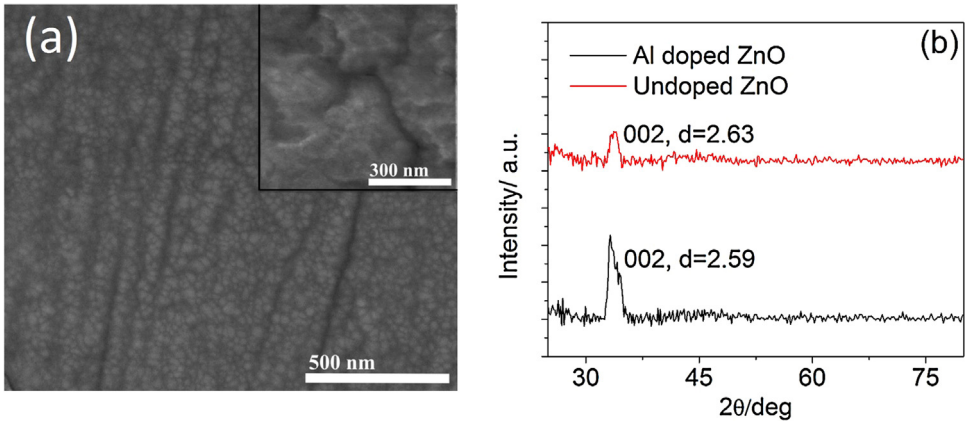

Fig. 1. (a) SEM morphology of the nanostructured AZO film, Inset shows ZnOfilms;(b) 2θ XRD scans of the ZnO and AZO films.

doping of ZnO has a wide application in gas and humidity sensors [12,13].

Effect of doping on the immobilization of enzyme and their activity remains largely unexplored. Changes in the surface charge due to introduction of the dopants might result in altered quantity of the enzyme immobilized on the surface and consequently the overall activity of the system.

Conventional electrode supports such as gold and Pt are expensive and difficult to fabricate [14]. Most of the electrodes involving the use of metal oxides have expensive indium tin oxide (ITO) as base electrode with mediators [15–17]. The major drawback in using ITO is indium migration during operation [18]. Therefore, there is a need for alternative materials as electrode support, with advantageous properties. Saha and Gupta reported Al- and Fe co-doped ZnO conductive biosensors on glass, avoiding the need for a support electrode [14]. In this work, we have used polycarbonate (PC) plastic as a base material to grow thin metal oxide films as compared to conventional ITO base [15]. PC features high optical transparency (90%), temperature compatibility (145 °C), low-water absorption (0.2–0.35%) and relatively low cost. The deposition of high quality films needs to be carried out around 100 °C because the glass transition temperature of PC is close to 145 °C. In addition PC is an easily maneuverable, light weight material which can be cut to exact required dimensions. The pulsed laser deposition (PLD) technique meets this requirement. PLD allows room/low temperature deposition of crystalline ZnO/AZO materials, produces low energy plasma particles that do not damage the plastic substrates and maintains the stoichiometry of the initial target material [19,20].

Glucose oxidase (GOx) is a widely studied redox protein used extensively for the fabrication of glucose sensors. It is a dimer of 160 kDa with flavin adenine dinucleotide (FAD) as the co-enzyme [21]. GOx presents two possible electron transfer mechanisms, one through the breakdown of the by-product hydrogen peroxide and the other through the direct electron transfer from $FADH_2$. The mechanism at play varies depending upon the distance between the catalytic site on the surface of the electrode and the mediator used in the buffer [22]. Because of these well-known properties, GOx is an ideal model enzyme for studying the electron transfer pathway for new material interfaces such as ZnO/PC. Especially, ZnO has a high iso-electric point of 9.3, which provides a suitable interface for GOx with an iso-electric point of 4.5 [2]. As the GOx electron transfer mechanism depends on the distance between the catalytic site and the surface charges on the electrode, it was interesting to investigate how this may influence the reactivity of GOx immobilized on ZnO/PC or AZO/PC to glucose without a mediator.

In this work high quality transparent and conductive ZnO and AZO films were obtained by PLD deposition on PC. The deposits were optimized by using different Al concentration and oxygen pressure conditions. The change in GOx activity on immobilization on the doped surface was explored. Since PC based ZnO films can perform as inexpensive, flexible and disposable electrodes, their usefulness for biosensing was validated by carrying out glucose tests with blood serum samples.

2. Experimental DetailsExperimental details

2.1. Thin films deposition and characterization

ZnO and AZO thin films were prepared by PLD using a high-power, Q-switched, frequency-quadrupled, Nd:YAG laser. The laser specifications were 266 nm wavelength, 150 mJ laser energy, 2 J cm^{-2} average fluence, 10 Hz repetition rate and 6 ns pulse width [13]. Rectangular sheets of 1 cm × 2 cm and 1.2 mm thick polycarbonate (Lexan 9030) were used as substrates. Prior to deposition the substrates were cleaned with isopropyl alcohol and then dried in nitrogen gas. The deposition chamber was pumped down to the base pressure of 3×10^{-5} mTorr ($\sim 4 \times 10^{-6}$ Pa) for all the depositions. The oxygen pressure in the chamber was kept at 10 mTorr (~1.3 Pa) and 30 mTorr (~4 Pa) for the ZnO and AZO thin films respectively. The substrate temperature was raised to 100 °C and kept constant for the whole deposition. Ten thousand laser shots were used for the 15 min long depositions, after which the substrate temperature was lowered to 30 °C.

The surface morphology was studied by scanning electron microscopy (FEG Quanta 400F). XRD was used to establish the crystalline structure of the samples using Bruker Discover D8. Optical transmission spectra were recorded using Jasco UV/VIS/IR spectrophotometer with a scan rate of 200 nm min^{-1} and band width of 2 nm. Surface reflectometry was carried out at an angle of 70° and a wavelength of 628 nm (JA Woolman surface reflectometer).The electrical properties such as sheet resistance and carrier concentration were measured using a four-point probe/Hall effect instrument (Accent HL5500).

2.2. Glucose oxidase immobilization

A stock solution of 2 mg mL^{-1} of GOx was prepared in phosphate buffer (pH 7.2). This solution (2.5 μL) was dropped on a 0.25 cm^2 (0.5 cm × 0.5 cm) area of a thin film of size 0.35 cm^2 (0.7 × 0.5 cm). To ensure uniform spreading, a 0.5 cm × 0.5 cm coverslip was placed on top of the drop. The solvent was allowed to evaporate at 4 °C for 24 h. The unbound enzyme was removed by rinsing with distilled water.