Sensors and Actuators B 137 (2009) 566–573

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Effect of nanostructure on the urea sensing properties of sol–gel synthesized ZnO

S.G. Ansari[a,d], Rizwan Wahab[a], Z.A. Ansari[c], Young-Soon Kim[a], Gilson Khang[b], A. Al-Hajry[d], Hyung-Shik Shin[a,*]

[a] School of Chemical Engineering, Chonbuk National University, Jeonju 561-756, Republic of Korea
[b] Department of Polymer Nano Science and Technology, Chonbuk National University, Jeonju 561-756, Republic of Korea
[c] Center for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India
[d] Department of Physics, Faculty of Science, Najran University, Najran, Saudi Arabia

ARTICLE INFO

Article history:
Received 9 September 2008
Received in revised form 1 January 2009
Accepted 9 January 2009
Available online 21 January 2009

Keywords:
Zinc oxide
Solution synthesis
Enzyme immobilization
Urea sensor
Surface composition

ABSTRACT

Urea sensing properties of zinc oxide in thick film form are presented here. Zinc oxide thick films were prepared on Al-sheet by conventional doctor blade method using organic additives. Flower-like structure and nanobelts of ZnO was synthesized by solution method using zinc acetate dihydrate and sodium hydroxide. Structural morphology significantly changed with precursor concentration (0.3–0.5 M) from a belt to flower-like structure. Urease was covalently attached with zinc oxide (by soaking in urease solution containing 100 units for 3 h). In general, conductivity of film increases after urease immobilization. The urease immobilized films were found sensitive to urea concentration from 1 mM to 100 mM. Three different sensitivity regions are observed viz. (i) lower concentrations (below 10 mM); (ii) linear region up to 50 mM; and (iii) a saturation region above 50 mM. Sensors are extremely sensitive in region (i). Nanobelt structure of ZnO resulted in highest urea sensitivity. Sensors exhibited linear response when tested for different concentration of human urine in buffer solution (1:9 to 4:6). The sensor responses are reproducible, reliable, reversible and selective, with a response time of 6 s.

1. Introduction

The human senses are the best examples of specialized neural sensors. Enzymatic electrochemical biosensors have attracted researcher's attentions due to their potential applications and improvement in performance with nano-technological approach. In last few decades, the development of enzyme-based biosensors has been a topic of considerable interest due to their potential applications, since the spectroscopic methods are laborious and often not useful in online monitoring system. The inconvenience was overcome by the use of electrochemical methods for biosensing.

The most widespread example of a commercial biosensor (third generation sensor) is the blood glucose biosensor, which uses an enzyme to breakdown the blood glucose, breaking the sugar down into its metabolites. Glucose oxidase (GOx) is a dimeric protein which catalyzes the oxidation of beta-D-glucose into D-glucono-1,5-lactone which then hydrolyzes to gluconic acid. In this process, it transfers an electron to the electrode which is used as a measure of the blood glucose concentration [1–7].

Among a large number of enzymes used for biosensor construction, urease is an important part in most enzyme-based sensor development to fulfill the growing demand for urea detection. Urea ($(NH_2)_2CO$) is basically an organic compound of carbon, nitrogen, oxygen and hydrogen. Most organisms' deal with the excretion of nitrogen waste originating from protein and amino acid catabolism. The normal level of urea in serum is 3–7 mM (15–40 mg/dl). In patients suffering from the renal insufficiency, the urea concentration in serum is from 30 mM to 80 mM (180–480 mg/dl) and at the level above 180 mg/dl, the hemodialysis is required. However, too high concentration in the blood can cause damage to body organs. Therefore, its analysis is of considerable importance.

An important part for biosensors construction is to immobilize biomolecules on the transducer without changing their structural conformation and their activity. The immobilization feature can govern the performance and reliability of the obtained biosensor. The host material generally used for biosensor development includes clays, layered double hydroxides (e.g. ZnAl), nanoporous alumina membranes, polymers, tin oxide, etc. [8–16]. Researchers are putting extensive effort in exploring/understanding the biocompatibility of inorganic (especially metal oxide) nanomaterials synthesized by various techniques. There are few reports that have shown the promising development of the third generation biosensors using such materials [17–20]. Nanomaterials offer unique advantages in immobilizing enzymes due to increase surface reactivity, preserving enzyme activity due to the microenvironment.

Zinc oxide; a versatile semiconductor, which has attracted attention for its wide range application in the field of solar cells, luminescent, electro-acoustic devices, etc. Being a nanoporous

* Corresponding author. Tel.: +82 63 2702438; fax: +82 63 2702306.
E-mail address: hsshin@chonbuk.ac.kr (H.-S. Shin).

0925-4005/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2009.01.018

material from the family of the richest nanostructure, it can also be used as a matrix for immobilization of biomolecules. ZnO has great potential for biosensing due to its biomimetic and fast electron transfer properties. High isoelectric point of ZnO (IEP ~9.5) makes it suitable for adsorption low IEP proteins such as uricase with IEP ~4.3, urease with IEP ~5.9, etc. Liu et al. [18] synthesized flower-like structure hydrothermally and used it as a matrix for horseradish peroxidase immobilization. Zhao et al. [19] used cross-linking method to immobilize GOx on nanoclustors of ZnO:Co films. Similarly, Zhang et al. [20] immobilized uricase on ZnO nanorods for uric acid sensing. However, in these reports, a complicated method was used to immobilize the protein. Here, we report a simple method to immobilize the protein on the nanostructured zinc oxide and construction of sensor in a thick film form. The suggested test method is simple and can be easily adopted to fabricate a probe.

A systematic study on the detection of urea and diluted-urine using urease immobilized zinc oxide is presented here. ZnO thick films were prepared on Al-sheet using doctor blade technique. Effect of urea/urine on the electrical properties of urease immobilized ZnO films were studied by varying potential across the film and measuring the corresponding current.

2. Experimental

2.1. Materials and methods

Zinc acetate dihydrate [$Zn(CH_3COO)_2{\cdot}2H_2O$] was used as a precursor with sodium hydroxide (NaOH) solution. Aluminum (99.999%, Goodfellows, size: $1 \times 1.5 \times 0.1\,cm^3$) was used as substrate. Urease (EC 3.5.1.5, from jack bean, 50 U/mg) and Urea (ACS reagent, 99.0–100.5%) were purchased from Sigma–Aldrich. Deionized (DI) water (resistivity of 18 MΩ, Milli-Q system, Millipore Inc.) was used for rinsing and preparing all aqueous solutions.

2.2. ZnO synthesis, thick film preparation and characterization

For synthesis of nano-structured ZnO, the solution method was used, wherein zinc acetate dihydrate (0.3–0.5 M) and 3 M sodium hydroxide was dissolved in 100 ml DI water, stirred well and refluxed at 90 °C for 1 h each. After refluxing, the precipitate (white powder) was neutralized with methanol and dried at room temperature.

A thick film paste was prepared mixing ZnO (70%) and organic additives (30%, ethyl cellulose (Biochemica) and Carbetole acetate (Aldrich) in agate-mortal and pastel. This paste was partially applied on electropolished Al-sheet (1 cm × 1.5 cm), using doctor blade method. Films (area: 1 cm^2) were allowed to settle at room temperature and then dried under IR-lamp (250 W). Film area was kept constant for all the samples prepared for the measurement. The substrates were degreased with acetone and electropolished prior to film deposition [21].

Morphological observations of powder and thick film samples were carried out by field emission scanning electron microscopy (FESEM, Hitachi S4700). Elemental and compositional analysis was carried out using X-ray diffraction spectrometry (XRD, Rigaku, Cu Kα). Urease immobilization was confirmed by obtaining surface state information using X-ray photoelectron spectroscopy (XPS, VG Microtech, U.K.), with Al Kα (15 kV, 20 mA) as X-ray source.

2.3. Enzyme immobilization

Urease was immobilized by soaking the samples in urease solution. Initially samples were immersed in a phosphate buffer solution (PBS, 0.1 M, pH 7.2) containing 0.2 mg of urease per ml for 3 h at 25 °C. Total 10 ml of solution was prepared containing 100 units of urease. The samples were then washed and kept in PBS until use. Amount of enzyme immobilized on these samples was not calculated or estimated.

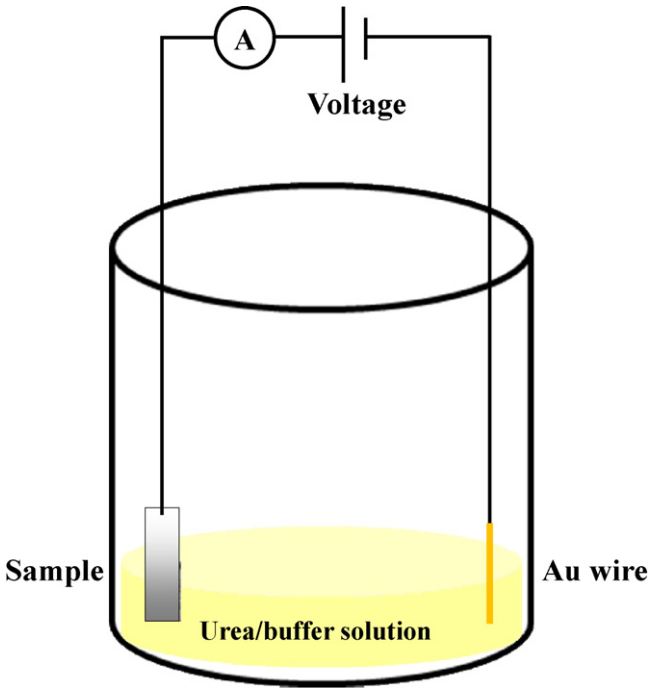

Fig. 1. Schematic of the cell constructed for urea sensing measurement; consists of gold wire (1 mm diameter, 5 cm length) as one electrode and urease immobilized films as another electrode.

2.4. Determination of enzyme activity after immobilization

After urease immobilization, the electrical property of ZnO thick films was studied to determine the enzyme activity. For this, potential from 0 V to 1 V was applied to the film and the corresponding current was measured using computer interfaced Keithely 6517/A electrometer. For each sample, five sets of measurement were carried out. A sample to sample (in a batch) was studied to understand the reliability of the sensors. All the measurements were carried out at room temperature (25 °C).

For this measurement, a cell, schemated as Fig. 1, was constructed consisting of gold wire (1 mm diameter, 5 cm length) as one electrode and urease immobilized ZnO films were used as another electrode. Urea solution prepared in PBS was used as an electrolyte. Amount of electrolyte was kept constant as 10 ml for all measurement. Solution was prepared with various concentrations of urea such as 1 mM, 3 mM, 5 mM, 10 mM, 20 mM, 50 mM and 100 mM (6–600 mg/dl). For this solution preparation, the amount of PBS was kept constant as 10 ml while the molar concentration of urea was calculated with reference to 10 ml of PBS. The respective amount of urea was added in separate beakers. Since the amount of urea with respect to PBS has changed with increasing molar concentration, this effect would reflect in the sensitivity curves indicating the variation due to urea, as presented below. Solution pH was neither measured nor controlled. The ratio of current to voltage is used as a measure of enzyme activity.

Human urine sample diluted with buffer solution from 1:9 to 4:6 (i.e. a total 10 ml of solution was prepared where urine concentration was varied from 1 ml to 4 ml).

Electrochemical properties were studied with WonAtech Potentiostat (WPG-100) at room temperature in a conventional three-electrode system. ZnO thick films were used as working electrode, platinum wire (1 mm dia) was used as auxiliary electrode