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Urea sensor based on tin oxide thin films prepared by modified plasma enhanced CVD

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Abstract

Urea sensing properties of tin oxide thin films are presented here. Tin oxide thin films were deposited by modified plasma enhanced chemical vapor deposition (CVD) technique at the deposition temperatures of 500–800 °C and RF power of 100 W. Film morphology significantly changed with deposition temperature from flakes to tiny crystals having tetragonal rutile structure grown along the [1 1 0] direction. Urease was covalently attached with tin oxide (by soaking in urease solution for 3 h). In general, conductivity of film increases after urease immobilization. The urease immobilized films were found sensitive to urea concentration from 1 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). Films deposited at higher temperature resulted in increased urea sensitivity. From the elemental analyses of the films after urease immobilization, urease was found attached with tin oxide, as evident by N 1s peak in the photoelectron spectra. A possible sensing mechanism is presented and discussed.

Keywords: Tin oxide; Chemical vapor deposition (CVD); Plasma processing and deposition; Enzyme immobilization; Urea sensor; Surface composition

1. Introduction

The human senses are the best examples of specialized neural sensors. A biosensor is an analytical device that detects the concentration of substances and their biologically parameters using specific biochemical reactions mediated by the biologically sensitive materials such as isolated enzymes, immunosystems, tissues, organelles or whole cells (i.e. biological receptor) by means of various transducers such as electrical, thermal or optical signals devices. The most widespread example of a commercial biosensor is the blood glucose biosensor, which uses an enzyme to breakdown the blood glucose. In this process, it transfers an electron to the electrode and this is used as a measure of blood glucose concentration [1–7]. Researchers and developers are putting extensive efforts in this field since the market demand for such sensors is growing fast and huge. There will be a great expansion potential for the associated sensor technologies for the development of such novel sensor devices in near future.

In last few decades, the development of enzyme-based biosensors has been a topic of considerable interest due to their potential applications. 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. In aquatic organisms the most common form of nitrogen waste is ammonia, while land-dwelling organisms convert the toxic ammonia to either urea or uric acid. 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 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 organs of the body. Therefore, its

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analysis is of considerable importance in agro-food chemistry and environmental monitoring.

An important part in biosensors construction is the immobilization of biomolecules on the transducer. The immobilization feature can govern the performance and reliability of the obtained biosensor [8–10]. The host material generally used for biosensor development includes clays, layered double hydroxides (e.g. ZnAl), nanoporous alumina membranes, polymers, etc. [8–13]. The role of inorganic materials needs to be studied in the development of biosensors due to their thermal stability, chemical inertness with biomolecules, low temperature operation, small size and possible integration/construction in the form of a tiny device. Large surface area can offer improved sensitivity and better performance allowing rapid analysis in vivo with the possibility of integration for device fabrication in the form of an electrochemical sensor or biosensor or biochip. The sensors based on these materials can be far better than those conventional spectroscopic determinations as these can be part of the on-line monitoring system, low power consumption and economical.

Tin oxide; a suitable candidate that generally finds wide application in the field of sensors, opacifiers, solar cells, etc. Its low cost, chemical stability; useful electrical properties made it a favorable industrial compound. However, application of this material needs to be explored for biosensing, which might add a new material in the class of bioinorganic materials [15–19].

In this paper, we present a systematic study on the detection of urea using urease immobilized tin oxide thin films. SnO_2 films were deposited at various temperatures (from 500 to 800 °C) using modified plasma enhanced chemical vapor deposition (CVD) system. Hydrated stannic chloride was used as a precursor and O_2 was used as a reactant gas for the deposition of tin oxide. Effect of urea on the electrical properties of urease immobilized tin oxide films were studied by varying potential across the film and measuring the corresponding current.

2. Experimental

2.1. Materials and methods

Hydrated $SnCl_4$, xH_2O (Junsei Chemicals, Japan) was used as precursor for deposition of tin oxide films. 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 for preparing all aqueous solutions.

2.2. SnO_2 thin film preparation and characterization

Silicon wafers with (1 0 0) orientation were used as substrate which were cleaned using conventional cleaning process prior to thin film deposition. Substrates were loaded at the plasma downstream, i.e. exhaust side. Precursor was loaded in alumina boat at the gas inlet side. Precursor and substrates were separately heated in vacuum (10^{-3} Torr). A RF coil was placed in between the precursor and substrates and plasma was generated using inductively coupled RF-power supply (13.56 MHz). Precursor was maintained at 90 °C. After the precursor and substrate reached to the required temperature, reactant gas, i.e. O_2 (99.99% pure) was flown at 300 sccm during deposition. Deposition was carried out at a pressure of 0.5 Torr for 15 min each from 500 to 800 °C at a constant plasma power of 100 W. The detailed synthesis conditions and procedures are reported elsewhere [15].

Morphological observation of thin film samples was 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) and using S-Probe ESCA model 2803 (Fision Instrument, 10 kV, 20 mA) with Al Kα as X-rays source.

2.3. Enzyme immobilization

Urease was immobilized by dipping the samples in solution. Initially samples were immersed in a pH 7.5 phosphate buffer (0.1 M) solution 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 with DI water and kept in phosphate buffer solution (at pH 7.5) until use. Amount of enzyme immobilized on these samples was not calculated or estimated.

2.4. Determination of enzyme activity after immobilization

After urease immobilization, the electrical property of SnO_2 thin films was studied to determine the enzyme activity. For this, potential from 0 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. All the measurements were carried out at room temperature (25 °C). Solution pH was neither measured nor controlled.

For this measurement, a cell was constructed consisting of gold wire (1 mm diameter, 5 cm length) as one electrode and urease immobilized SnO_2 films were used as another electrode. Urea solution prepared in DI water 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, 3, 5, 10, 20, 50 and 100 mM (6–600 mg/dl). The ratio of voltage and current is used as a measure of enzyme activity.

3. Results and discussion

3.1. SnO_2 thin film characterization

FESEM examination shows the variation in the microstructure of SnO_2 film with growth temperature. Fig. 1a–d shows the FESEM images of samples deposited at 500, 600, 700 and 800 °C, respectively. A noticeable increase in grain size with deposition temperature is observed. At 500 °C (Fig. 1a) and 600 °C (Fig. 1b) small flakes are observed which grew in to the well-faceted tiny crystals of a micrometer in size at 700 °C (Fig. 1c) and 800 °C (Fig. 1d). At the same time, the porosity of the film has increased with increasing deposition temperature.