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Highly stable urea sensor based on ZnO nanorods directly grown on Ag/glass electrodes

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ABSTRACT

Vertically aligned zinc oxide nanorods (ZnO NRs) were directly grown on Ag sputtered glass substrate by a low-temperature solution route. The fabricated urea sensor (glass/Ag/ZnO NRs/urease) showed a high sensitivity of 41.64 μA/mM cm^2, wide linear range 0.001–24.0 mM, and low detection limit of 10 μM. The low value of the Michaelis–Menten constant $\left(K_M^{app} = 0.328 \text{ mM}\right)$ indicates the high affinity of urease towards the urea. The directly synthesized ZnO NRs on the electrode expressively increases NRs attachment and its specific surface area. These features further results in high enzyme loading and enhanced sensor efficiency. The urea sensor showed an excellent anti-interference ability against electroactive species.

1. Introduction

Accurate, reliable, selective, and easy to operate quantification devices are the basic requirements of several industries including biomedical, pharmaceutical, cosmetic, food etc. Being specific towards clinical and agricultural investigations, urea has a great significance as an end product of nitrogen metabolism and crucial indicator of liver and kidney function [1,2]. An increased urea level in blood (normal level is 2.5–7.5 mM) and urine causes renal failure (acute or chronic), urinary tract obstruction, dehydration, shock, burns, and gastrointestinal bleeding [3,4]. On the other hand, a low urea concentration executes hepatic failure, nephritic syndrome, cachexia (low-protein and high-carbohydrate diets) [5]. Hence, it is of utmost importance to develop novel and cost effective diagnosis techniques for real time monitoring of urea in physiological fluids (urine and blood). Although many methods has been studied for urea estimation such as gas chromatography [6], calorimetry [7], and fluorimetry [8], but they require complicated sample pretreatment steps and are unsuitable for on-site monitoring. Resolving these issues a number of transducers are introduced for urea detection, i.e., amperometric, potentiometric, optical, thermal, and piezoelectric [9–14].

Urease-based amperometric sensors, owing to low detection limit, high selectivity, and sensitivity with potential ability for real-time and on-site analysis, are the most favorite subjects amongst researchers. In amperometric biosensors, smart innovative matrices are critical to an effective response [15–19]. Where, they not only provide support but also impart stability to biomolecules towards variations in temperature, pH, and ionic strength, thereby increase its self-life and reduce fabrication cost. In this regard, many matrices such as polymers, sol–gels, conducting polymers, Langmuir–Blodgett films, nanomaterials, and self-assembled monolayers (SAMs) have been employed for urea sensor fabrication [20–25]. Recently nano-metal oxides have emerged as a new class of biosensor matrices because of its low cost, easy fabrication, and flexibility in tuning desired features such as an optimum surface-to-volume ratio, high catalytic efficiency, and an ability to adsorb biomolecules [26–28]. Especially, nanostructured zinc oxide (ZnO) was observed to be highly advantageous for several low isoelectric point (IEP) enzymes immobilization and improving sensor performances due to high IEP (9.5), strong adsorption ability, high specific surface area, wide band gap (3.37 eV), biocompatibility, and high electron communication features [29–33]. Along with choosing the nano-ZnO as desirable matrix, most studies were focused not only on the use of expensive electrodes (gold, glassy carbon, platinum, etc.) but also grown nanostructures on different substrates, separated and transferred them onto electrodes,

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which needs additional attention for preparation of nanostructures containing solution, coating, network-forming for proper adhesion, and drying.

Herein, we report vertically aligned ZnO nanorods (NRs) directly synthesized on glass/Ag electrode by a low-temperature solution process. Thereafter immobilization of urease enzyme was performed to fabricate high performance urea sensor (glass/Ag/ZnO NRs/urease). Additionally, the anti-interference ability of urea sensor was studied employing interfering species such as glucose, uric acid, cholesterol, lactic acid, and ascorbic acid.

2. Experimental details

2.1. Reagents

Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$, 99%), hexamethylenetetramine (HMTA, 99%), Jack bean urease type III (EC 3.5.1.5 from *Canavalia ensiformis*), urea, cholesterol (water soluble), glucose (d-(+)-99.5%), uric acid (UA), ascorbic acid (AA), lactic acid (LA), sodium phosphate monobasic anhydrous (NaH_2PO_4), sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$), and sodium chloride (NaCl) were purchased from Sigma–Aldrich and used without further purification. Phosphate buffer saline solution (PBS; 50 mM, pH 7) was freshly prepared by mixing solutions of NaH_2PO_4, $Na_2HPO_4{\cdot}2H_2O$, and NaCl (0.9%) in deionized water prior to the experiments. Enzyme solutions were prepared by dissolving 1 mg/mL urease in PBS solution.

2.2. ZnO NRs synthesis and characterization

In a typical synthesis process, an equimolar (0.03 M) of $Zn(NO_3)_2{\cdot}6H_2O$ and HMTA was dispersed in distilled water (50 mL); transferred into Pyrex glass bottle with suspended seeded substrate upside down; and heated at 85 °C for 5 h. After the completion of reaction, the electrodes were rinsed with DI water to remove impurities. The morphology and crystallinity of as-synthesized ZnO NRs were examined by field emission scanning electron microscopy (FESEM, Hitachi S4700), transmission electron microscopy (TEM, JEOL-JEM-2010), and X-ray diffractometer (XRD) measured with Cu-Kα radiations (λ = 1.54178 Å) in the range of 30–60° with 8°/min scanning speed.

2.3. Fabrication of urea sensor and measurements

The systematic fabrication process of the bioelectrode (glass/Ag/ZnO NRs/urease) for urea sensing was shown in Fig. 1. Firstly, a thin layer of Ag (~120 nm) was sputtered onto the glass substrate (1 × 0.5 cm) followed by deposition of ZnO seed layer (40–50 nm) on sputtered Ag (0.25 cm^2) (i and ii). In the next step, vertically aligned ZnO NR arrays were grown on the seeded region as described above (iii). Prior to the urease immobilization, the bioelectrode was rinsed with PBS in order to generate hydrophilic surfaces. Then 10 μL of urease solution was immobilized on to the ZnO NRs surfaces by physical adsorption and dried at room temperature for over-night (iv). In order to remove unbound enzyme, the device was further rinsed with DI water for several times. Electrochemical measurements were conducted by cyclic voltammetry (CV) connected to personal computer. All experiments were carried out using a conventional three-electrode system with the fabricated bioelectrode as the working electrode, platinum wire as the counter electrode, and Ag/AgCl with saturated KCl solution as the reference electrode. All the potentials in these experiments were measured with respect to Ag/AgCl reference electrode and the electrochemical measurements were carried out at room temperature in PBS. Each CV measurements were performed in a solution of 10 ml volume between −0.20 and +0.80 V (vs. Ag/AgCl).

3. Results and discussion

3.1. Morphological characterization of ZnO NRs

Fig. 2 shows the top view (a) and cross-sectional (b) FESEM images of as-grown ZnO NRs. Uniform and vertically aligned ZnO NRs perpendicularly to the substrate were observed in high density. From the TEM image of as-grown single ZnO NR (c), the NR length is around 1.6 μm with a base and tip diameter of ~20 and ~10 nm, respectively. The SAED pattern suggests that the ZnO NRs growth is along [0 0 0 1] direction, the polar *c*-axis of ZnO crystal lattice (inset in c). The high resolution TEM (HRTEM) clearly delineates the fringes of ZnO with interplanar spacing of about 0.52 nm confirming its single crystallinity (d). Fig. 3(a) shows the purity single crystalline of ZnO NRs characterized by XRD. This confirms that the ZnO NRs belong to hexagonal wurtzite ZnO phase with a strong intensity of (0 0 2) peak, indicating preferential growth along the *c*-axis direction. No other impurity peaks were detected. Further, abundant urease immobilized on ZnO NRs was clearly noticed by the captured FESEM image (Fig. 3(b)).

3.2. Electrochemical studies

A schematic of fabricated bioelectrode (glass/Ag/ZnO NRs/urease) with urea sensing mechanism is shown in Fig. 4(a).

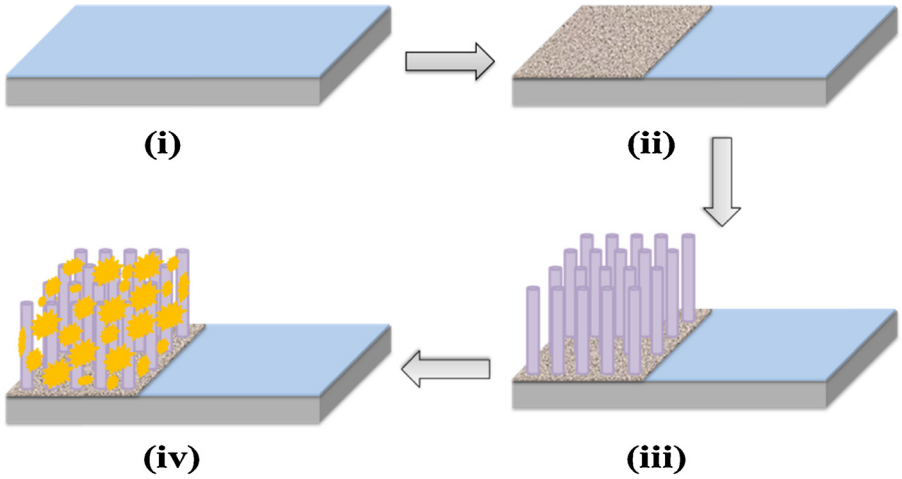

Fig. 1. Systematic fabrication steps of the bioelectrode (glass/Ag/ZnO NRs/urease): (i and ii) Ag and ZnO seed layer deposition by sputter, (iii) growth of ZnO NRs, and (iv) urease immobilization.