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A promising biosensing-platform based on bismuth oxide polycrystalline-modified electrode: Characterization and its application in development of amperometric glucose sensor

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ABSTRACT

Nano-structured bismuth oxide (nano-BiO_x) is a suitable material for enzyme immobilization owing to its attractive properties, such as large specific surface area, suitable permeability of the resulting film, the high biocompatibility, and as well as photovoltaic effect from semiconductor nanoparticles. Thus, a new type of amperometric glucose biosensor based on nano-BiO_x was constructed. The amperometric detection of glucose was assayed by potentiostating the GOD/nano-BiO_x electrode at 0.5 V to oxidize the enzymatically generated hydrogen peroxide. The proposed biosensor provided a linear response to glucose over a concentration range of 1×10^{-6} M to 1.5×10^{-3} M with a sensitivity of 51.0 ± 0.4 mA/(M cm^2) and a detection limit of 4×10^{-7} M based on S/N = 3. The apparent Michaelis–Menten constant was calculated to be 2.9×10^{-3} M. In addition, characterization of nano-BiO_x and modified electrode was performed by FT-IR spectroscopy, Raman spectroscopy, scanning electron microscope (SEM) and rotating-disk electrode (RDE) voltammetry.

1. Introduction

Bismuth oxide (BiO_x) is an interesting material, very important in modern solid-state technology and fascinating to scientists, owing to its unique structures and physical properties like the large energy band-gap, high refractive index, dielectric permittivity and high oxygen-ion conductivity, as well as marked photoconductivity and photoluminescence [1–3]. These special features of BiO_x make it suitable for a large range of applications, such as gas-sensors, optical coatings, photovoltaic cells and microwave integrated circuits [4–6]. In addition, BiO_x is nontoxic and has excellent chemical inertness and biocompatibility.

Since the discovery of carbon nanotubes [7], nano-structured-materials have received intensive research interest due to their unique properties and potential applications in, for example, nanoscale electronics, optoelectronics, and magnetics [8]. Nano-sized bismuth oxide (nano-BiO_x) shows greater advantages and novel characteristics than regular sized particles, such as the much higher specific surface and greater surface free energy, which are favorable for the biomolecules adsorption. Encouraged by these properties of nanoscaled-material, in this work, we aim to continuously extend the application of nano-BiO_x into the bioelectrochemical research area to construct a suitable platform for biosensing. Structural and morphology characterization, FT-IR, Raman scattering and SEM studies performed on the synthesized nano-BiO_x are presented. The permeation property of the nano-BiO_x modified electrode was investigated by rotating-disk electrode experiment (RDE). Since glucose oxidase (GOD) is well-studied, inexpensive, stable and practically applied in clinical and chemical analyses [9], this enzyme was used as a model enzyme to explore the ability of the synthesized nano-BiO_x as a matrix for enzyme immobilization in the design of an amperometric glucose biosensor.

2. Experimental

2.1. Reagents

Glucose oxidase (GOD) (EC 1.1.3.4, Type II, 108 U/mg) from *Aspergillus niger* was purchased from Amresco. Nanostuctured bismuth oxide (nano-BiO_x) samples were synthesized according to an earlier reported procedure [10,11]. All other chemicals were of analytical grade and used without further purification. Phosphate

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buffer solutions (PBS) were 0.1 M Na_2HPO_4 and NaH_2PO_4 and its pH was adjusted with H_3PO_4 or NaOH solutions, twice-distilled water was used throughout the experiment. Fifty millimolars stock glucose solution was daily prepared and stored overnight to reach mutarotational equilibrium before use.

2.2. Construction of GOD/nano-BiO_x electrode

The nano-BiO_x developed glucose sensor was prepared as follows: The nano-BiO_x colloidal suspension (2 mg/ml) was prepared by dispersing nano-BiO_x in deionized water with stirring overnight. GOD was also dissolved in deionized water with a concentration of 4 mg/ml. A defined amount of aqueous GOD/nano-BiO_x mixtures (containing 30 μg GOD, 15 μg nano-BiO_x) was spread on the surface of the working electrode. The mixture was dried at room temperature, leading to a nano-BiO_x film in which enzymes were entrapped. The resulting electrode was placed in saturated glutaraldehyde vapor at room temperature for 15 min in order to induce the chemical cross-linking of the entrapped enzyme molecules.

2.3. Instrumentation

A CHI 660 electrochemical workstation (CHI Co., USA) was used for electrochemical experiments. All electrochemical studies were performed with a conventional three-electrode system. A saturated calomel electrode (SCE) and a Pt foil electrode were used as the reference electrode and the counter electrode, respectively. The working electrode was a platinum disk electrode (diameter 2 mm), which was polished carefully with 0.05 μm alumina particles on silk and was then rinsed with distilled water and dried in air before use. Fourier transform infrared (FT-IR) spectra of the samples were measured on a pressed pellet with KBr, employing a Tensor 27 spectrometer (Bruker, Canada). A micro-Raman spectrometer (Jobin Yvon Horiba T64000) was employed to obtain Raman scattering spectra. Micrographs were obtained with a XL-30E SEM (Philips, Netherlands).

3. Results and discussion

3.1. Characteristics of synthesized nano-BiO_x

As shown in Fig. 1A, B, it can be seen that the surface of the prepared BiO_x was composed of numerous nanostructures, dominantly, nano-sized rods and sheets. The diameter of every nanorod is about 44 nm (Fig. 1C), and the thickness of nanosheet is about 95 nm. IR property of the as-prepared sample was investigated. Absorption bands between 400 and 600 cm^{-1} due to vibrations of Bi–O bonds [12]; 810 cm^{-1} ascribed to vibration of Bi–O–Bi [13]; besides, a very strong absorption band at about 1380 cm^{-1} were observed (Fig. 1D). The results of X-ray powder diffraction (XRD) identified that the synthesized bismuth oxide nanomaterials used in this work were composed of mixed phase of monoclinic α-Bi_2O_3, body-centred cubic γ-Bi_2O_3, BiO, nonstiochiometric $Bi_2O_{2.3}$ along with the presence of bismuth oxide nitrate hydroxide hydrate [11], which are crucial for further surface functionalization and coating by any other functional

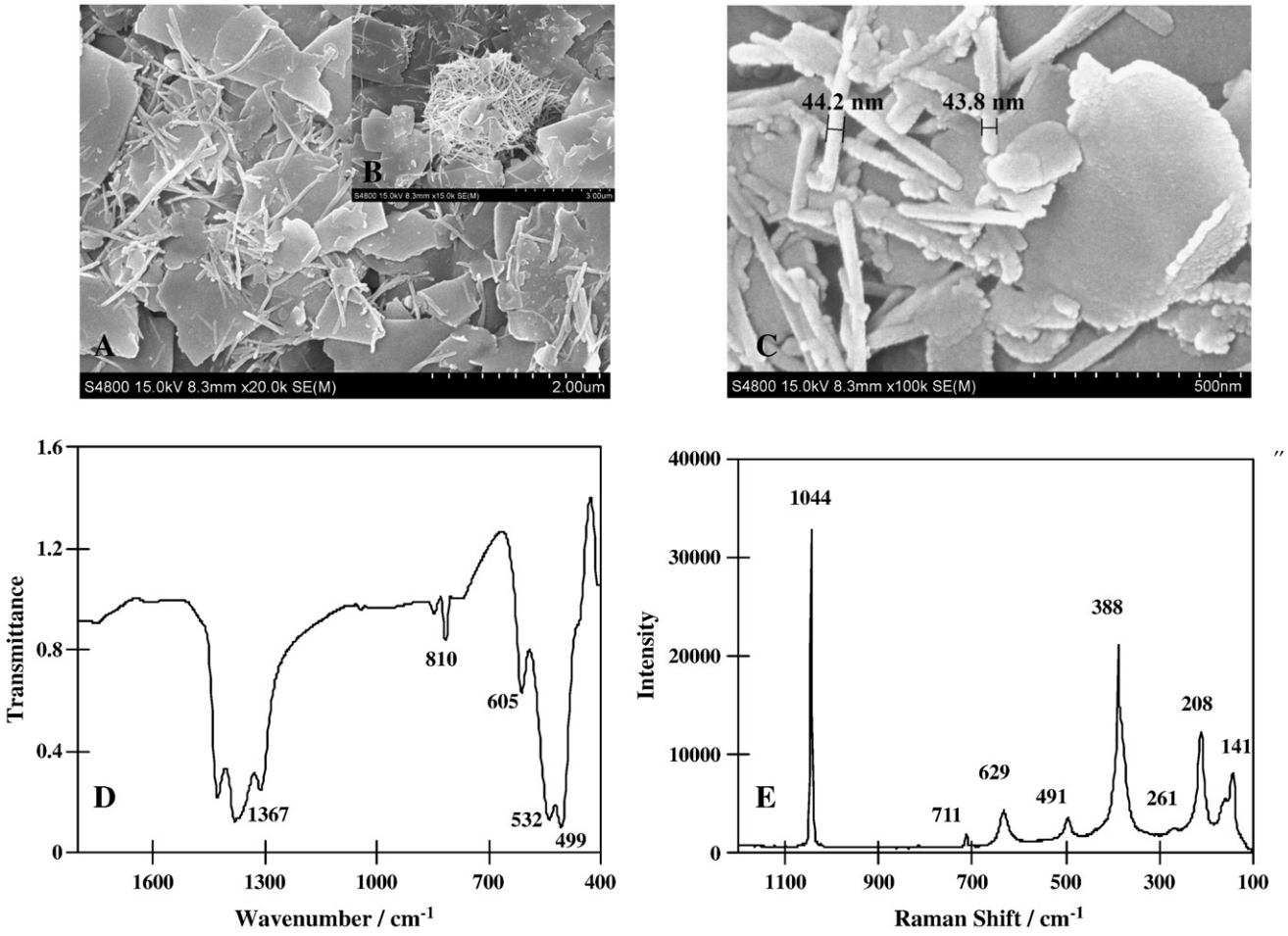

Fig. 1. Surface morphologies of as-synthesized BiO_x: (A) and (B) low-magnification SEM images and (C) high-magnification SEM image, FT-IR spectrum (D), Raman scattering spectrum (E).

group. In addition, Fig. 1D reveals that the prepared polycrystalline bismuth oxides have strong IR absorption, which can result in good optoelectronic property. The Raman spectrum of the 1D BiO_x nanostructures shows seven Raman bands at 141, 208, 388, 491, 627, 711 and 1044 cm^{-1} (Fig. 1E). The four Raman bands observed at 141, 208, 388 and 491 cm^{-1} are expected to be due to the Bi–O stretches representing (A_g, B_g) modes of Bi_2O_3 [14]. The prepared BiO_x shows a broad Raman band compared to those of 1D nanowire or nanoflowers [6].

The permeation property of nano-BiO_x modified electrode was determined by rotating-disk electrode experiments (RDE) that were carried out at different rotation rates in the presence of hydroquinone (2 mM) in 0.1 M PBS (pH 6.5) (Fig. 2A). The permeability (P_m) was determined from Eq. (1)

$$\frac{1}{i_{lim}} = \frac{1}{0.62nFAD_s^{2/3}C^0\nu^{-1/6}\omega^{1/2}} + \frac{1}{nFC^0AP_m}. \quad (1)$$

Where i_{lim} (A) is the steady-state limiting current, A (cm^2) is the electrode area, D_s (cm^2/s) corresponds to the diffusion coefficients of the substrate in the bulk solution, while C^0 ($mol\,l^{-1}$) is the hydroquinone concentration, ν (m^2/s) is the kinetic viscosity of the solution, and ω (rpm) is the rotation rate of the RDE. Experimental data from RDE voltammograms were conveniently displayed in the form of Koutecky–Levich plots of the reciprocal of the limiting current vs. reciprocal of the square root of the rotation rate. The data relative to the nano-BiO_x coating presents a linear behavior with the same slope as for a bare electrode with positive intercept whose value depends on the permeability P_m (cm/s) of the film (Fig. 2B). P_m of nano-BiO_x film was estimated to be 2.3×10^{-2} cm/s, which is comparable to that of polypyrrole-alginate film (2.7×10^{-2} cm/s) [15] and smaller than that of alginate film (3.65×10^{-1} cm/s) [15]. The reasonable permeability of enzyme immobilization matrix based-film leads to the high and efficient mass transfer of the reactants and good accessibility of the immobilized enzymes, inducing the rapid, sensitive and stable response to enzyme substrate [16,17].

3.2. Analytical performance of the GOD/nano-BiO_x modified electrode

Thus, this attractive nano-BiO_x was used to immobilize GOD for the construction of a novel glucose sensor. GOD catalyses, in the presence of molecular oxygen, the oxidation of β-D-glucose into gluconic acid and hydrogen peroxide. As a consequence, the amperometric detection of glucose was assayed by potentiostating the GOD/nano-BiO_x electrode at 0.5 V to oxidize the enzymatically generated hydrogen peroxide. Fig. 3 shows the typical response curves for the GOD/nano-BiO_x modified platinum electrode on successive injections of glucose to the stirring PBS (0.1 M, pH 6.5). Inset A in Fig. 3 depicts the corresponding calibration curve. Current density is defined as $j = i/A$ (i is current and A is normal surface area of GCE). The biosensor achieves 95% of the steady-state current within 5 s, with a linear range of 1.0×10^{-6} to 1.5×10^{-3} M. The detection limit is 4×10^{-7} M based on S/N = 3. The sensitivity is calculated to be 51.0 ± 0.4 mA/(M cm^2). The apparent Michaelis–Menten constant (K_M^{app}) can be determined from the Lineweaver–Burk equation, Eq. (2)

$$\frac{1}{I_{ss}} = \frac{K_M^{app}}{I_{max}}\frac{1}{C} + \frac{1}{I_{max}}. \quad (2)$$

Where I_{ss} is the stationary current, I_{max} is the maximum response current, C is the concentration of the substrate. Thus, K_M^{app} was calculated to be 2.9 mM by the analysis of the slope and the intercept of Lineweaver–Burk plot (inset B of Fig. 3). The proposed GOD/nano-BiO_x electrode possesses superior analytical performance to other glucose biosensors based on semiconductor nanoparticles, for instance, GOD/TiO_2 [18], GOD/ZrO_2-Chitosan [19], and GOD/ZnO [20]. As is known, nano-BiO_x belongs to semiconductor nanoparticles, which are photosensitive materials. Moreover, BiO_x owns wider band-gap (2.58–2.85 eV) [21]. When bismuth oxide nanomaterials are exposed under solar, they may generate electrons and holes, respectively, in the conduction and valence bands, which can be used for the substrate reduction (oxidation) through electron accepter (donor) [22]. Thus, this special photovoltaic effect of semiconductor nanoparticles may play an assistant function in enzymatic reaction, making the catalysis of enzyme more efficiently and contributing to the higher sensitivity and reactivity.

The immobilized enzyme with nano-BiO_x exhibited also dramatically enhanced thermodynamic stability and life span, which may be ascribed to the beneficial effect brought by nano-BiO_x as the enzyme host matrix. The optimal temperature for glucose biosensing was 53 °C (Fig. 4). This value is greater than those commonly observed for GOD entrapped in other inorganic materials, namely 40, 45 and 50 °C for cryohydrogel, zeolite matrix and inorganic laponite clay, respectively [23–25]. Such an increase of the optimal temperature can be

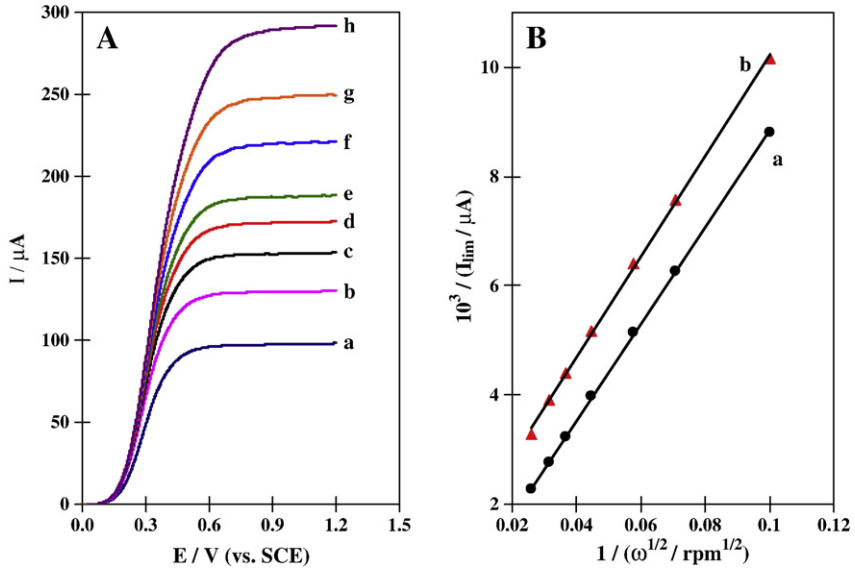

Fig. 2. (A) Rotating-disk electrode voltammograms of nano-BiO_x modified disk electrode (diameter 4 mm) in the presence of 2 mM solution of hydroquinone in 0.1 M PBS (pH 7): (a) 100, (b) 200, (c) 300, (d) 400, (e) 500, (f) 750, (g) 1000 and (h) 1500 rpm. Scan rate: 10 mV/s. (B) Koutecky–Levich plots for bare electrode (a) and nano-BiO_x modified electrode (b).

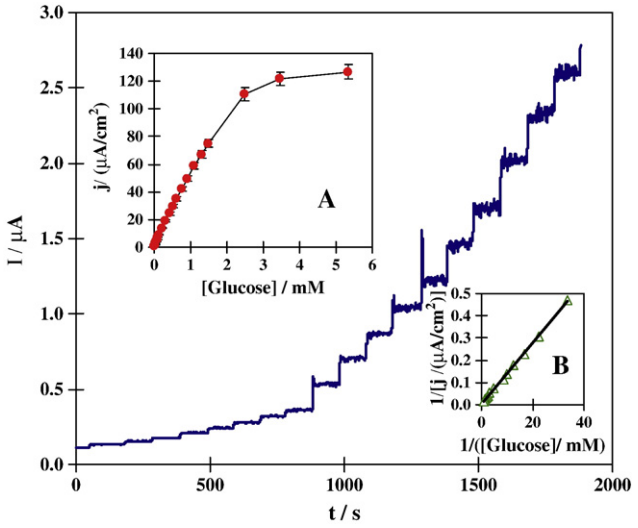

Fig. 3. Current–time responses of GOD/nano-BiO_x modified electrode to successive addition of glucose solution. Applied potential: 0.5 V; stirring rate $\omega = 300$ rpm; temperature: 25 °C; initial working volume: 15 ml; supporting electrolyte: 0.1 M PBS, pH 6.5. Inset A: Calibration curve of the proposed electrode to glucose; Inset B: The Lineweaver–Burk plot obtained with the proposed electrode.

attributed to two factors: One is due to the cross-linking of biospecies through glutaraldehyde and the conformational effect through a lowering of degrees of freedom for the cross-linked enzyme resulting in stability of enzyme [26]. Another is assigned to the utilization of nano-BiO_x as enzyme host matrix. The immobilization matrix prevents the enzyme from thermal denaturation. Partial enzymes can be encapsulated or embedded in nano-BiO_x during the procedure of enzyme immobilization. With the special "shell" of nano-BiO_x, the encapsulated or embedded enzymes are possible to exhibit the characteristic of thermal resistance. The dependence of current response on temperature range 5–53 °C can be expressed as an electrochemical version of the Arrhenius relationship with Eq. (3):

$$\ln i(T) = \ln i_0 - \frac{E_a}{RT}. \quad (3)$$

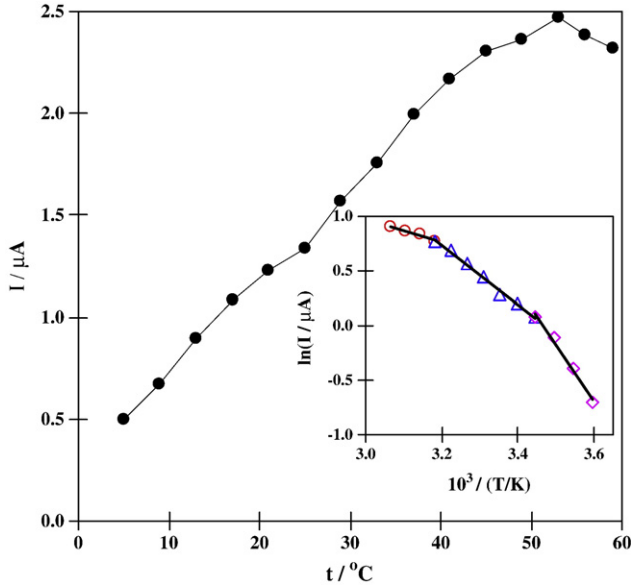

Fig. 4. Influence of temperature on biosensor sensitivity. Inset: The Arrhenius plot of ln*j* against 1/*T*.

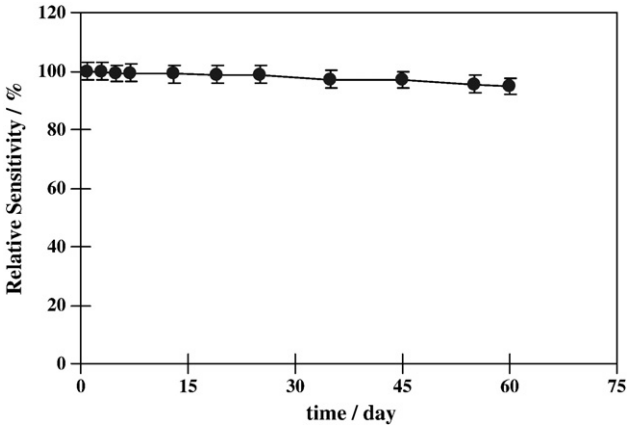

Fig. 5. Long-term stability of the proposed GOD/nano-BiO_x electrode.

Where R is the gas constant in J/(mol K), T is the absolute temperature in Kelvin and E_a is the activation energy in kJ/mol. Uncommonly, the Arrhenius plots exhibit three straight lines that intersected at 41 and 17 °C, respectively (inset of Fig. 4). Such Arrhenius plots have already been reported for other GOD electrodes and have been ascribed to conformational changes of the immobilized enzyme with temperature [27]. The activation energies were calculated from the slope of three lines and were found namely to be 44.0, 22.5 and 9.0 kJ/mol. These values are quite different from those reported for the free enzyme, namely 14.63 kJ mol^{-1} [28]. Nevertheless, the value 22.5 kJ mol^{-1} was in good accordance with those commonly encountered for immobilized GOD (22.4–50 kJ mol^{-1}).

The storage stability of the biosensor was investigated by periodical measurement of its response to glucose. It is seen that no significant loss of activity of the immobilized enzyme during investigated period; it retained about 94.7% of its original response even after 60 days (Fig. 5).

4. Conclusion

It has been shown that polycrystalline of nano-BiO_x combines a number of positive features, as large specific surface area, reasonable permeability of the resulting film, the high biocompatibility and its particular properties of semiconductor, which make it prospective material platform for the biosensors. Further enhancement of the sensitivity of the biosensor based on nano-BiO_x might be achieved by employing single crystal, for example δ-Bi_2O_3. The latter possesses the superior conductivity [29].

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