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Bismuth oxide nanorods based immunosensor for mycotoxin detection

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ABSTRACT

We report results of the studies relating to fabrication of an efficient immunosensor based on bismuth oxide nanorods (nBi_2O_3), electrophoretically deposited onto indium-tin-oxide (ITO) coated glass substrate. This immunosensor was fabricated by immobilization of anti-aflatoxin monoclonal antibodies (Ab-AFB1) and bovine serum albumin (BSA) for aflatoxin B1 detection. The structural and morphological studies of n-Bi_2O_3 have been carried out by XRD, UV–vis spectrophotometer; SEM, AFM and FTIR. It was found that the nBi_2O_3 provided improved sensing characteristics to the electrode interface in terms of electroactive surface area, diffusion coefficient, charge transfer rate constant and electron transfer kinetics. The results of electrochemical response studies of this BSA/Ab-AFB1/nBi_2O_3/ITO immunosensor revealed good linearity in the range of 1–70 ng dL^{-1} with low detection limit of 8.715 ng/dL, improved sensitivity of 1.132 μA/(ng/dL cm^{-2}), regression coefficient R^2 of 0.918 and reproducibility of >11 times. The association constant for the BSA/Ab-AFB1/nBi_2O_3/ITO immunosensor was determined as 7.318 ng/dL.

1. Introduction

The nanostructured metal oxides have recently aroused much attention of researchers actively engaged in different scientific disciplines including materials science, bioscience and nanotechnology due to their interesting properties and potential applications [1,2]. In the past decade, several researches on the synthesis of low-dimensional nanostructures are underway [3,4]. Their attractive properties and a wide range of applications have been attributed to their interesting structures. Bismuth and its compounds are fascinating to scientists due to their unique structures and properties [5] such as highly anisotropic Fermi surface, high refractive index, dielectric permittivity and low carrier densities [6,7]. Therefore, it has been widely used for the development of solid oxide fuel cells, gas sensors, photoelectric materials, high temperature superconductor materials, catalysts, functional ceramics [8–11]. Recently, 1D metal oxides have been found to display high surface basicity, fast oxygen ion mobility, efficient charge transfer ability and interesting catalytic properties [12,13]. These properties have been predicted to be useful for investigating the effect of both dimensionality and size on physical properties, and also for application in nanodevices [3]. Bismuth oxide (Bi_2O_3) based compounds are known to be better solid electrolytes than those of zirconia since the face-centred cubic (fcc) Bi_2O_3 has been found to exhibit the highest ion conductivity of all oxide ion conductors.

Bi_2O_3 is one of the important metal oxides since it plays a significant role in modern solid-state technology due to its peculiar properties such as wide band gap, high refractive index, high dielectric permittivity, high oxygen conductivity, resistivity, photoconductivity and photoluminescence, etc. Its excellent properties have been found to have a variety of applications such as catalysts, optical coatings [14], photovoltaic cells, microwave integrated circuits, fuel cells [15], nanoenergetic gas generator [16] and in the fabrication of sensors [17,18]. Moreover, they have been predicted to provide a favorable environment for the biomolecules adsorption, nontoxicity, chemical inertness, biocompatibility, greater surface free energy, and good electrochemical stability, etc. [19,20]. Recently, Bi_2O_3 film has been utilized for the detection of phenolic compounds using amperometric biosensors [21]. A composite of Bi_2O_3 with chitosan has been used for the fabrication of an electrochemical DNA biosensor [18]. Ding et al. have fabricated a nano-Bi_2Ox electrode for glucose detection by amperometric technique [19]. A nanocomposite of Bi_2O_3-multi walled carbon nanotubes (MWCNTs) onto glassy carbon electrode (GCE) has utilized for hydrogen peroxide detection [20]. A lithium doped bismuth oxide - MWCNTs modified GCE has found application in monitoring electrocatalytical behavior of ascorbic acid [22,23].

The contamination of food by mycotoxins is currently a matter of great concern, as these are responsible for many diseases [24,25]. Worldwide, about 25% of crops are annually affected by toxigenic fungi. Thus, mycotoxins have become a part of the food chain. High levels of mycotoxins in the diet can cause adverse acute and chronic effects on humans and animals. These may particularly affect liver, kidney,

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nervous system, endocrine and immune systems [26]. Aflatoxin B1 (AFB1) is one of the most potent toxic substances as compared to other types of aflatoxins i.e. B_2, B_3, G_1 and G_2 that occur naturally. AFB1 is known to be carcinogenic, mutagenic, immunosuppressive agents that are a group of closely related mycotoxins produced by fungi *Aspergillus flavus* and *A. parasites* in fruit, vegetable and food product [27]. The aflatoxins may cause contamination of nuts (almonds, walnuts), cereals (rice, wheat, maize) and oilseeds (soybean and peanuts). Some of the conventional methods currently being used for mycotoxins detection include thin layer chromatography liquid chromatography, high-performance liquid chromatography, immune-chromatography, enzyme-linked immunosorbent assay [28,29]. These techniques are time consuming, expensive, and require high skilled manpower, sometimes may give unreliable results for food and biological samples due to the difficulty in obtaining a clear solution for analysis. Thus, there is an urgent need for the development of reliable, rapid, quick and sensitive method for detection of aflatoxins. Kalita et al. have fabricated ring like nickel nanoparticles (RnNi) NPs of 10–20 nm through pulsed laser ablation method for an electrochemical immunosensor for AFB1 detection. This bioelectrode shows the linearity as 5–100 ng dL^{-1}, sensitivity of 0.59 μA/ng dL^{-1} with detection limit of 32.7 ng dL^{-1} [30]. Singh et al. used samarium oxide nanorods modified ITO electrode for the co-immobilization of Ab-AFB1. This fabricated immunoelectrode (BSA/Ab-AFB$_1$/n-Sm_2O_3/ITO) showed the detection of AFB1 in a range of 10–700 pg mL^{-1} with sensitivity and detection limit of 48.39 μA pg^{-1} mL^{-1} cm^{-2} and 57.82 pg mL^{-1} cm^{-1}, respectively [31]. The microelectrode was fabricated using 2-aminoethane thiol (AET) on Au electrode and used for fabrication of immunosensor by self assembling of horseradish peroxidase (HRP) and AFB1 antibody molecules for AFB1 detection [32]. The linear range was found to be from 0.5 to 10 ng mL^{-1} with a relatively low detection limit of 0.1 ng mL^{-1}. Glassy carbon electrode (GCE) consisting of chitosan/Au nanoparticle/anti-AFB$_1$, and a Fe_3O_4 magnetic core with a gold shell functionalized with 3-((2-mercaptoethylimino) methyl) benzene-1,2-diol for the detection of AFB1 [33]. This immunoelectrode revealed the detect range of 0.6–110 ng mL^{-1} with a detection limit of 0.2 ng mL^{-1} for AFB1. Polythionine (PTH)/AuNP modified GCE based electrochemical immunosensor exhibited a linear range of 0.6–2.4 ng mL^{-1} AFB1 and a limit of detection of 0.07 ng mL^{-1} AFB1 for the determination of aflatoxin B1 [34].

We report results of studies relating to the preparation of Bi_2O_3 nanorods *via* sol-gel method for development of an immunosensor for aflatoxin AFB1 detection. It is shown that the Ab-AFB1/nBi_2O_3/ITO bioelectrode exhibits improved sensitivity, low detection limit and wide detection range (Table 1).

2. Materials and methods

2.1. Materials

Bismuth nitrate [$Bi(NO_3)_3 \cdot 5H_2O$], polyethylene glycol (PEG), nitric acid (HNO_3) were purchased from MERCK (New Delhi, India). Aflatoxin B1 (AFB1), anti-aflatoxin B1 mouse monoclonal antibodies (Ab-AFB1) and bovine serum albumin (BSA, 98%) were procured from Sigma-Aldrich (USA). All other chemicals were of analytical grade and used without further purification. The indium-tin-oxide (ITO) coated glass (Balzers) sheet of resistance 15 W/cm was used as substrate for deposition of the desired nanocomposite that worked as the working electrode. All solutions in these studies were prepared using deionized water of resistivity of not less than 18 MΩ taken from a Milli-Q water purification system (Milli-Q, USA).

2.2. Preparation of antibody aflatoxin B1 (Ab-AFB1) and bovine serum albumin (BSA) solutions

The stock solution of AFB1 was freshly prepared in phosphate buffer (50 mM, pH 7.0) with 10% methanol and aliquoted in different working concentrations (1–70 ng dL^{-1}). Ab-AFB1 was prepared in phosphate buffer (50 mM, pH 7.0) prior to being used. 0.15 M sodium azide (NaN_3) was added into both these solutions as a preservative and stored at −20 °C when not in use. The bovine serum albumin (BSA, 98%) was freshly prepared (2 mg mL^{-1}) in phosphate buffer (50 mM, pH 7.0) and used as blocking agent for non-specific binding sites.

2.3. Synthesis of Bi_2O_3 nanorods

In a typical procedure, 0.05 mol $Bi(NO_3)_3$ was dissolved into 30 mL of 0.05 mol/L HNO_3 to get transparent Bi^{3+} acidic solution. 20 mL polyethylene glycol (PEG-600) was added drop wise as a dispersant to the above solution and magnetically stirred for 2 h at room temperature. 50 mL of NaOH (4 mol) aqueous solutions was quickly poured into it under vigorous stirring resulting in a yellow solution. The resultant yellow solution was stirred at 90 °C for 2 h. Then it was filtered and washed with deionized water and ethanol for 5–10 times. The precipitation was dried at 70 °C for 12 h in vacuum drier to obtain faint yellow Bi_2O_3 nanorods.

2.4. Preparation of nBi_2O_3 films by electrophoretic deposition

Electrophoretic deposition (EPD) was carried out by using a DC battery (BioRad, Model 200/2.0). Prior to deposition of nBi_2O_3 onto ITO substrate, uniform suspension of known amount of nBi_2O_3 (10 mg) was prepared in de-ionized water (1 mg mL^{-1}) through vigorous stirring followed by sonication for about 1 h. During EPD, a platinum foil (1 cm × 2 cm) was used as the anode and a hydrolyzed ITO coated glass plate as cathode. The two electrodes, placed parallel to each other with separation of 1 cm, were dipped in the nBi_2O_3 colloidal suspension. The colloidal suspension solution consisted of positively charged nBi_2O_3 nanoparticles. Since ITO plates were hydrolyzed, OH group were present on its surface. The optimized condition for EPD of Bi_2O_3 nanorods onto ITO plates was obtained as 10 V for 20 s as the nBi_2O_3 film exhibits maximum amperometric current at this condition. During the process positively charged Bi_2O_3 nanorods were attracted with OH group present on ITO surface *via* electrostatic interaction. Thus nBi_2O_3 (0.25 cm^2) was deposited onto ITO plate. This nBi_2O_3/ITO electrode was washed with deionized water followed by ethanol to remove any unbound particles and dried at room temperature (25 °C) for 12 h.

2.5. Fabrication of immunoelectrode

The optimal amount of freshly prepared solution (10 μL) of Ab-AFB1 was uniformly spread onto the desired nBi_2O_3/ITO electrode surface and

Table 1
Characteristics of the BSA/Ab-AFB1/nBi_2O_3/ITO immunoelectrode along with those reported in the literature for aflatoxin B1 detection, with their important parameters.

Bioelectrode	Detection method	Sensitivity	Limit of detection	Detection range	Stability (days)	Ref.
RnNi-film/ITO	Electrochemical	0.59 μA/ng dL^{-1}	32.7 ng dL^{-1}	5–100 ng dL^{-1}	60	[30]
BSA/anti-AFB1/MWCNTs/ITO	Electrochemical	95.2 mA ng/mL cm^{-2}	0.08 ng mL^{-1}	0.25–1.375 ng mL^{-1}	45	[31]
BSA-anti-AFB1/Au NPs	Conductometric	–	10.1 ng mL^{-1}	0.5–10 ng mL^{-1}	12	[32]
GCE/chitosan/AuNP/aAFB1	Electrochemical	–	0.2 ng mL^{-1}	0.6–110 ng mL^{-1}	–	[33]
PTH/AuNP/GCE	Electrochemical	–	0.07 ng mL^{-1}	0.6–2.4 ng mL^{-1}	–	[34]
BSA/Ab-AFB1/nBi_2 O_3/ITO	Electrochemical	1.132 μA ng/dL cm^{-2}	8.72 ng dL^{-1}	1–70 ng dL^{-1}	15	Present work