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A highly sensitive amperometric immunosensor probe based on gold nanoparticle functionalized poly (3, 4-ethylenedioxythiophene) doped with graphene oxide for efficient detection of aflatoxin B_1

Arati Sharma[a], A. Kumar[a,*], Raju Khan[b,*]

[a] Materials Research Laboratory, Department of Physics, Tezpur University, Tezpur 784028, Assam, India
[b] Analytical Chemistry Division, CSIR-North East Institute of Science & Technology, Academy of Scientific and Innovative Research, Jorhat 785006, Assam, India

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ABSTRACT

In the present work, a platform of Poly (3, 4-ethylenedioxythiophene) (PEDOT) and graphene oxide (GO) composite decorated with spherical gold nanoparticles (AuNPs) has been developed for rapid electrochemical detection of aflatoxin B1 (AFB_1). Electrochemical deposition of EDOT onto glassy carbon electrode (GCE) keeping GO as dopant followed by introduction of AuNPs has been achieved resulting in nanohybrid AuNPs/PEDOT-GO electrode. The antibody anti-aflatoxin B1 (anti-AFB1) has been further covalently immobilized onto the surface of AuNPs/PEDOT-GO using EDC/NHS coupling. The morphological and surface characteristics have been studied using Field Emission Scanning Microscopy (FESEM) and contact angle measurements. Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) studies have been carried out to investigate the electro-catalytic behavior of the modified electrodes. The heterogeneous rate constant (k_s) and transfer coefficient (α) have been determined by using Laviron's method. The proposed immunosensor exhibits a very high sensitivity of 0.989 μA ng mL^{-1} and 0.397 μA ng mL^{-1} within two linear range of 0.5–20 ng mL^{-1} and 20–60 ng mL^{-1}, respectively.

1. Introduction

Aflatoxins B1 are the most commonly found mycotoxins that are considered most hepatotoxic and hepatocarcinogenic classified into group I by the International Agency for Research on Cancers. They are difuranocoumarin derivatives produced by a polyketide pathway by many strains of Aspergillus flavus and Aspergillus parasiticus that are common contaminant in agriculture [1]. They are associated with many crops including peanuts, corn, cottonseed, Brazil nuts, pistachios, spices, copra (dried coconut) and figs particularly in hot and humid regions of the world [2]. Some recent works on enzyme based biosensors have been reported towards the detection of aflatoxin B1 and achieved good sensitivity [3,4]. Several classical analytical methods have been developed for the detection and quantification of aflatoxins in agricultural products and processed food products. Based on the principle of detection the methods can broadly be grouped into chromatographic viz. thin-layer chromatography (TLC) [5], high-performance liquid chromatography (HPLC) and gas chromatography (GC) [6], spectroscopic viz. ultraviolet (UV), diode array detection (DAD), fluorescence detection (FD) or mass spectrometry (MS) [7] detectors, and immunochemical method like enzyme-linked immunosorbent assay (ELISA) [8]. The chromatographic and spectroscopic methods require highly qualified expertise and extensive sample purification [9]. Immunoassays are relatively simple and easy-to-use methods for detection of AFB1 in different food products in comparison to the chromatographic methods. However, the enzymes used as labels may suffer from the instability due to their denaturation and degradation resulting in false positives or false negatives in ELISA [10].

Inexpensive immunosensor has been long pursued to detect AFB1 for food quality screening but the sensitivity is far from satisfactory. In this regard, cost-effective portable biosensors for efficient detection of AFB1 with high sensitivity and selectivity are required [11,12,13,14].

In the present work, conducting polymer Poly (3, 4-ethylene dioxythiophene) (PEDOT), based immunosensor has been developed. PEDOT can be controllably deposited onto a substrate surface by electro-polymerization of monomer EDOT by the application of potential [15] and it has been used in biological and biomedical areas such as biosensors and bio-interfaces [16,17]. The introduction of a thin conductive polymer film increases the effective surface area of the electrode substrate without sacrificing the conductive property of the electrode [18]. Moreover, it can easily be functionalized with negatively charged dopants like GO, functionalized CNTs, PSS and metal

* Corresponding authors.
E-mail addresses: ask@tezu.ernet.in (A. Kumar), rajukhan@neist.res.in (R. Khan).

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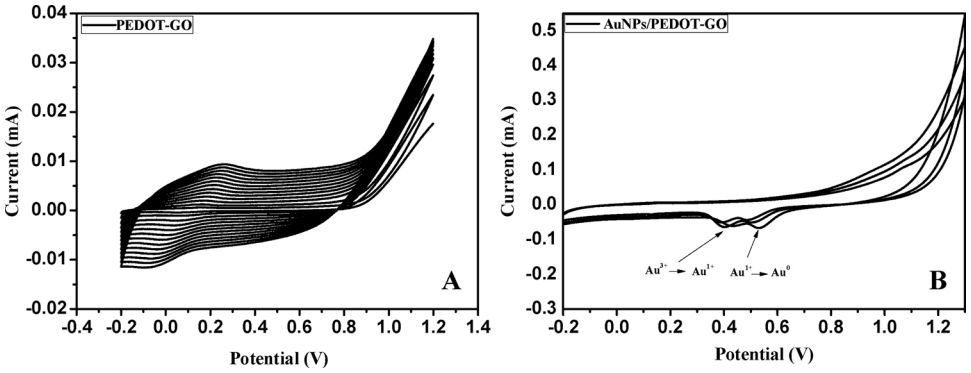

Fig. 1. Cyclic Voltammogram pattern of electrochemical polymerization of (A) EDOT in presence of GO as dopant and (B) Deposition of Au-NPs onto PEDOT-GO/GCE.

nanoparticles as the electro-polymerization of the monomer results in positively charged polymer backbone [19,20]. The hydrophobic nature and the absence of functional groups such as −COOH & $-NH_2$ of this polymer may hinder its application in biosensors. *In situ* incorporation of GO in PEDOT layers has been carried out in order to get a hydrophilic surface with free functional groups for covalent binding with antibodies and the composite is formed by π-π stacking between the GO layers and polymer rings [21]. It has been reported that a highly efficient antibody or enzyme electrode can be obtained by covalent attachment of carboxyl group of GO with the amine groups of the proteins. The electrode offers broad linearity, excellent reproducibility and storage stability [22]. However, a GO based biosensor has sensitivity much lower than that of a graphene-based biosensor due to its poor conductivity [23]. In order to improve the sensitivity of the PEDOT-GO composite based electrodes, gold nanoparticles (AuNPs) have been deposited. Spherical gold nanoparticles (AuNPs) of 5 nm–100 nm size have drawn significant attention in the construction of immunosensors due to their high conductivity and improved immobilization ability [24,25,26]. AuNPs help in better immobilization of the protein molecules providing native microenvironment thereby retaining their activity and preventing them from leaching back into the bulk solution [27].

Although both PEDOT and GO are good substrate for depositing AuNPs to fabricate an efficient immunosensor, the loading of Au-NPs onto the composites has been reported. The synergistic effect of all the components may provide an excellent platform for immobilization of anti-AFB1 with improved analytical parameters.

In this study we have explored a sensitive and stable electrochemical immunosensing strategy for highly efficient and rapid detection of AFB1. The electrode has been synthesized by two-step procedure (i) electrochemical polymerization of EDOT in presence GO as dopant, (ii) deposition of AuNPs by electrochemical reduction of auric chloride onto the modified GCE. The bioelectrode has been designed with a view to enhance the sensitivity towards the detection of AFB1. The immunosensors show an excellent sensitivity of 0.98 μA ng mL^{-1}within a linear range of 0.5 ng mL^{-1} to 20 ng mL^{-1}.

2. Materials and methods

2.1. Chemicals and reagents

AFB_1, monoclonal anti aflatoxin antibody AFB_1, bovine serum albumin (BSA ≥ 98%), 3,4- Ethylenedioxythiophene (EDOT ≥ 97%), Graphene Oxide and gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$ ≥ 99%) are all procured from sigma Aldrich.

2.2. Instrumentation

Morphological studies were carried out by Field emission Scanning Electron Microscope (JEOL-JSM-6390LV). Contact angle measurements were made by Degree of Hydrophilicity measurement set up (Model: DSA 15B). All the electrochemical measurements, viz. cyclic voltammetry, impedance spectroscopy and Differential pulse voltammetry were performed using a Potentiostat/Galvanostat/ZRA (Gamry Reference 3000, USA) with Gamry Echem Analyst Software. Glassy carbon electrode, Ag/AgCl (3 M KCl) and a platinum wire were used as working, reference and auxiliary electrodes, respectively. The FT-IR spectrum (Spectrum 100 with software version CPU32) was used for the conformational studies.

2.3. Electrochemical synthesis of PEDOT-GO/GCE

PEDOT-GO composite has been prepared by electrochemical polymerization of EDOT in presence of GO as dopant. 50 μL of GO (10 mg/mL) was added to 10 mL d-H_2O in a vial containing 0.1 M EDOT and the solution was sonicated for 15 min. Prior to deposition, the solution was stirred for 6 h at a temperature below 5 °C. Electro-polymerization was carried out using cyclic voltammetry by adjusting the potential from −0.2 V to 1.2 V. The potential was cycled 13 times at a scan rate of 10 mV/s vs. Ag/AgCl and the result is shown in Fig. 1(A).

2.4. Electrochemical deposition of gold nanoparticles (AuNPs) layer on to PEDOT-GO/GCE

The synthesized PEDOT-GO/GCE was immersed in a solution containing 500 μL of 3 mM $HAuCl_4$ and 0.1 M KCl in a 10 mL vial. The Au-NPs were electrochemically deposited onto the PEDOT-GO electrode using CV by applying potential from −0.2 V to 1.2 V vs. Ag/AgCl at a scan rate of 10 mV/s and it shown in Fig. 1(B).

2.5. Immobilization of monoclonal anti-aflatoxin B1 antibody on to AuNPs/PEDOT-GO/GCE

The schematic representation of fabrication process of the immunosensor is shown in Scheme 1. After the GCE was modified with the AuNPs/PEDOT-GO/GCE, it was soaked in EDC (0.4 M) and NHS (0.1 M) for 30 min to activate the carboxyl group [28]. 10 μL of 5 μg mL^{-1} stock solution of monoclonal anti-AFB1 antibody in 100 mM phosphate buffer (pH 7.0) containing 0.05% Tween-20 was limited within the working electrode area and incubated for 5 h at 25 °C to form anti-AFB_1/AuNPs/PEDOT-GO/GCE. After rinsing with PBS, 10 μL of 5 mg mL^{-1} BSA solution was used to block the unspecified sites to prevent non-specific adsorption. Finally, the BSA/anti-AFB_1/AuNPs-PEDOT-GO/GCE was rinsed with PBS and stored at 4 °C when not in use.

2.6. Preparation of aflatoxin B_1 (AFB_1) solution and real sample

The standard AFB1 solution was prepared by dissolving it in 10% methanol and 90% PBS solution (1:9; v/v) and stored in 4 °C. Real Sample matrix is prepared by adding 5 g non infected corn powder with 10% methanol solution in PBS solution and mixed with 0.1 g NaCl [29]. The components are finely mixed in shaker for 30 min followed by