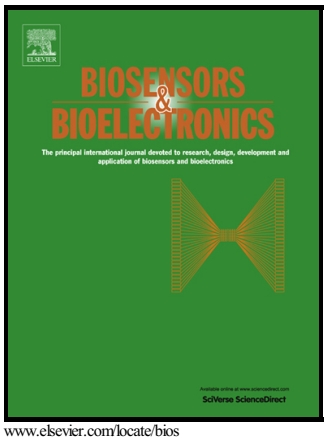

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Abstract

In this work, a novel electrochemical immunosensor was developed for the detection of botulinum neurotoxin-E (BoNT/E). This method relied on graphene nanosheets-aryldiazonium salt modified glassy carbon electrodes (GCE) as sensing platform and enzyme induced silver nanoparticles (AgNPs) deposited on gold nanoparticles (AuNPs) as signal amplifier. Herein, a GCE was electrografted with mixed monolayer of phenyl and aminophenyl (Ph-PhNH_2/GCE) by diazotization reaction. Further, graphene nanosheets (GNS) were covalently attached on electrode surface (GNS/Ph-PhNH_2/GCE). Field emission scanning electron microscopy (FE-SEM), X-ray diffraction (XRD), atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were employed to characterize synthesized graphene oxide and modified electrode surfaces. In the sandwich immunoassay format, the sensitivity was amplified using rabbit anti-mouse IgG-alkaline phosphatase (RαMIgG-ALP) functionalized with gold nanoparticles (RαMIgG-ALP/AuNPs). In order to study the immunosensing performance of GNS/Ph-PhNH_2/GCE, first the capturing antibody (rabbit-anti BoNT/E antibody) was covalently immobilized via EDC/NHS chemistry. Further, the electrode was sequentially subjected to sample containing spiked BoNT/E, revealing antibody (mouse-anti BoNT/E) followed by RαMIgG-ALP/AuNPs. 3-indoxyl phosphate (3-IP) was used as substrate which finally reduces the silver ions. The deposited AgNPs on electrode surface were determined by linear sweep voltammetry (LSV). The developed electrochemical immunosensor could detect BoNT/E with linear range of 10 pg/ml-10 ng/ml with the minimum detection limit of 5.0 pg/ml and total analysis time of 65 min. In addition, the immunosensor was successfully evaluated against food samples (orange juice and milk).