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Synthesis of highly dispersed zinc oxide nanoparticles on carboxylic graphene for development a sensitive acetylcholinesterase biosensor

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ABSTRACT

Highly dispersed zinc oxide nanoparticles (ZnO NPs) were synthesized on carboxylic graphene (CGR). A novel acetylcholinesterase (AChE) biosensor based on ZnO NPs, CGR and Nafion (NF) hybrids modified glass carbon electrode (GCE) has been successfully developed. ZnO NPs-CGR was homogeneously dispersed in NF and dropped on the surface of GCE. ZnO NPs-CGR-NF possessed excellent conductivity, catalysis and biocompatibility which were attributed to the synergistic effects of ZnO NPs, CGR and NF. ZnO NPs-CGR-NF/GCE provided a hydrophilic surface for AChE adhesion. The AChE biosensor showed favorable affinity to acetylthiocholine chloride (ATCl) and could catalyze the hydrolysis of ATCl with an apparent Michaelis-Menten constant value of 126 μM, which was then oxidized to produce a detectable and fast response. Under optimum conditions, the biosensor detected chlorpyrifos and carbofuran ranging from 1.0×10^{-13} to 1×10^{-8} M and from 1.0×10^{-12} to 1×10^{-8} M. The detection limits for chlorpyrifos and carbofuran were 5×10^{-14} M and 5.2×10^{-13} M, respectively. The developed biosensor exhibited many advantages such as good sensitivity, stability, reproducibility and low cost, thus providing a promising tool for analysis of enzyme inhibitors. This study could provide a universal platform for meeting the demand of the effective immobilization enzyme on the electrode surface.

1. Introduction

Pesticides are widely used in agriculture due to their high efficiency as insecticides. Unfortunately, these compounds exhibit high acute toxicity, with the majority being hazardous to both human health and the environment. Indeed, the inhibition of AChE activity by pesticides can lead to a disturbance of normal neuronal function and possibly death [1,2]. Therefore the exact and speedy measurement of pesticides in water and food is of great importance. Biosensors based on AChE have emerged as a promising technique for toxicity analysis, environmental monitoring, food quality and military investigations in recent years [3,4]. The main application of AChE biosensors is for the detection of organophosphate and carbamate pesticides based on enzyme inhibition. These devices are designed to complement or replace the existing reference analytical methods such as HPLC, GC, GC/MS and etc. by simplifying or eliminating sample preparation, thus decreasing the analysis time and cost. Our research purpose is to develop a sensitive and stable AChE biosensor for detection of pesticides to reach the same level of these analytical instruments.

Graphene, a two-dimensional sheet of sp^2-bonded carbon atoms arranged in a honeycomb lattice, has attracted increasing attention since it was first isolated from three-dimensional graphite by mechanical exfoliation [5]. Due to its extraordinary thermal, mechanical, and electrical properties, graphene is usually considered as a competitive candidate for next-generation electronic applications such as super-capacitors [6], batteries [7], sensors [8,9], biosensors [10,11], catalysts [12,13], etc. However, many researches have reported that the pure graphene actually exhibit unsatisfactory electrical conductivity because of the inevitable aggregation [14,15]. A useful method to prepare functionalized graphene is incorporated into chemical functional groups by covalent bonding on the graphene sheets. Some of the useful and unique properties of graphene can only be realized after it functioned with organic groups such as hydroxyl, carboxyl, amino and the like [14–16].

Nanostructured metal oxide semiconductors possess high surface area, nontoxicity, good biocompatibility, catalytic activity, chemical stability. They have been investigated for various applications such as solar cells, electrochemistry sensors and biosensor. Among the metal oxide semiconductors, ZnO NPs with the wurtzite crystal structure is an n-type semiconductor with a wide, direct band gap of 3.37 eV room temperature has been investigated for various applications such as photocatalysts [17,18], dye sensitized solar cells [19] and biosensor [20]. In recent years,

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the nanocomposite of ZnO NPs and graphene was synthesized and applied in the investigation fields of electrochemistry such as solar cells, photoluminescence, gas sensor, non-enzymatic hydrogen peroxide and glucose sensor, photocatalytic and antibacterial properties of graphene-ZnO NPs hybrids [21–24]. ZnO NPs were uniformly dispersed on functionalized graphene sheets and formed homogeneous ZnO NPs on functionalized graphene sheets. According to the results of investigation [14,15], we developed the AChE biosensor based on ZnO NPs-CGR-NF nanocomposites.

Nafion (NF) polymer is chemically inert, ideal conductivity, hydrophilic, and insoluble in water, and thus possesses almost ideal properties for preparation of modified electrodes [25]. Some nanomaterials with high conductivity and catalytic activity are combined with NF and used to modify electrode that seems to be a possible approach to improve the sensitivity, selectivity and stability of the modified electrode. For example, Kumaravel et al. reported that nanosilver/NF electrode for electrochemical detection of methyl parathion showed strong electro catalytic activity, good stability and reproducibility [26]. Li et al. presented that NF-graphene nanocomposite film modified electrode for determination cadmium exhibited high sensitivity [27]. Li et al. proposed that horseradish peroxidase biosensor based on NF/graphene electrode for determination of H_2O_2 exhibited both good operational storage and storage stability [28].

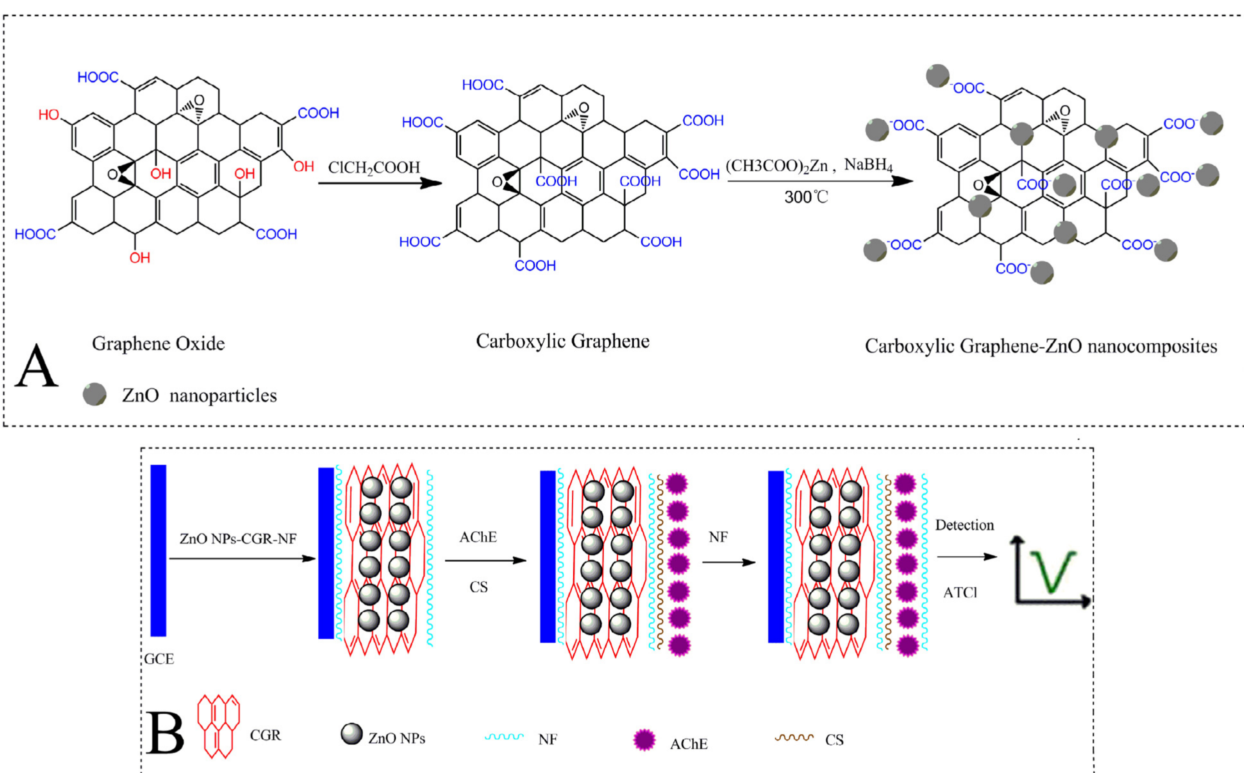
Chitosin (CS) is an abundant natural biopolymer with excellent film forming ability, biocompatibility and nontoxicity, which provides natural microenvironment to the enzyme and also gives sufficient accessibility to electrons to shuttle between the enzyme and the electrode [29].

Based on the above researches, the ZnO NPs-CGR nanocomposite was homogeneously dispersed in NF then dropped on the surface of GCE and formed uniform membrane. Combining the excellent characteristics of ZnO NPs, CGR and NF, a novel AChE biosensor based on ZnO NPs-CGR-NF/GCE was developed. The ZnO NPs-CGR-NF possessed excellent conductivity, catalytic activity and biocompatibility which were attributed to the synergistic effects of ZnO NPs, CGR and NF. Furthermore, ZnO NPs-CGR-NF/GCE provided hydrophilic surface for AChE adhesion. CS was used to immobilize AChE on the surface of ZnO NPs-CGR-NF/GCE to keep the AChE activities and assist electrons to shuttle between the enzyme and-CSNS-NF/GCE. Finally, NF was used as a protective membrane of the AChE biosensors to improve the stability of the biosensor. The biosensor exhibited excellent affinity to its substrate and the catalytic effect on the hydrolysis of ATCl. The biosensor has been demonstrated as a device with high sensitivity, acceptable stability and reproducibility for the analysis of ATCl and pesticides. More importantly, this study provides a universal platform for meeting the demand of the effective immobilization enzyme on the ZnO NPs-CGR-NF/GCE surface. The process of preparation ZnO NPs-CGR nanocomposites (A) and fabrication of the biosensor (B) was showed in Scheme 1.

2. Experimental

2.1. Chemicals

ATCl, AChE (Type C3389, 500 U/mg from electric eel), CS (85% deacetylation) and NF (5% in lower aliphatic alcohols and water) were purchased from Sigma-Aldrich (St. Louis, USA). Chlorpyrifos and carbofuran (99.99%) were obtained from AccuStandard (USA). Graphite powder was purchased from Sinopharm Chemical Reageat Company. (China). Bovine serum albumin (BSA) and $(CH_3COO)_2Zn{\cdot}6H_2O$ was obtained from Shanghai Chemical Reagent Co. Ltd. (China). All other reagents were of analytical grade. Aqueous solutions were prepared with deionized (DI) water (18 MΩ cm).



Scheme 1. The process of preparation ZnO NPs-CGR nanocomposite (A) and fabrication of the biosensor (B).