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Graphene modified screen printed immunosensor for highly sensitive detection of parathion

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ABSTRACT

Due to indiscriminate use of pesticides, there is a growing need to develop sensors that can sensitively detect the trace amount of pesticides in food and water samples. Parathion, identified as an acetylcholinesterase inhibitor, had been one of the most widely used pesticides throughout the world. Symptoms of its poisoning are found to be diverse enough to include nausea, vomiting, diarrhea, muscle cramping/twitching, and shortness of breath. In this work, a graphene based impedimetric immunosensor has been fabricated and employed for highly sensitive and specific detection of parathion. The fabrication proceeded through the modification of the screen-printed carbon electrodes (SPE) with graphene sheets, followed by their functionalization with 2-aminobenzyl amine (2-ABA) via an electrochemical reaction. These amine functionalized graphene electrodes were then bio-interfaced with the anti-parathion antibodies. In the impedimetric mode, this biosensor detected parathion in a broad linear range, i.e. 0.1–1000 ng/L with a very low limit of detection (52 pg/L). It also showed high selectivity towards parathion in the presence of malathion, paraoxon, and fenitrothion. The viability of this biosensor was demonstrated by detecting parathion in real samples (e.g., tomato and carrot) and through cross-calibration against HPLC.

1. Introduction

Different classes of pesticides, including organophosphates, organochlorides, carbamates, pyrethrums, and triazines are used extensively to combat agricultural pests (Aragay et al., 2012). Due to unsystematic use of these pesticides, their residues accumulate in the food chain and ultimately find their way to human consumers though water, food, and soil (Audrey et al., 2012). The organophosphates (OPs) are one of the most widely used classes of pesticides (approximately 36%) (Sukirtha and Usharani, 2013). Their toxicity effects in vertebrates are well documented (Aslan et al., 2011). The OPs are known to inhibit the activity of the essential acetylcholinesterase (AChE) enzyme, consequently posing various health risks, e.g. nerve disorders, respiratory diseases, and sometimes even death (Liu and Lin, 2005; Singh et al., 1999). Parathion is still recorded as one of the most commonly used OPs in developing counties like India (Consumer Resources, India, 2015). Therefore, a routine and a convenient detection of parathion is essential to maintain the quality of the food and water human consumes.

High performance liquid chromatography (HPLC) and gas chromatography–mass spectroscopy (GC–MS) are the common techniques for the qualitative and quantitative detection of pesticides (Du et al., 2010; Wen et al., 2010). The above methods are time consuming and costly while producing large amounts of organic waste. Hence, they are not necessarily preferable options for an on-field real time monitoring (Liu and Lin, 2005). During the last two decades, the biosensors have gained considerable analytical interest for the determination of pesticides due to advantages of portability, routine detection, and fast processing time (Mulchandani et al., 2001). Different biosensing platforms are categorized on the basis of the type of the bio-recognizing element (e.g., antibody, protein, enzyme, and microorganism) and or the transduction unit (e.g. optical, piezoelectric, and electrochemical)

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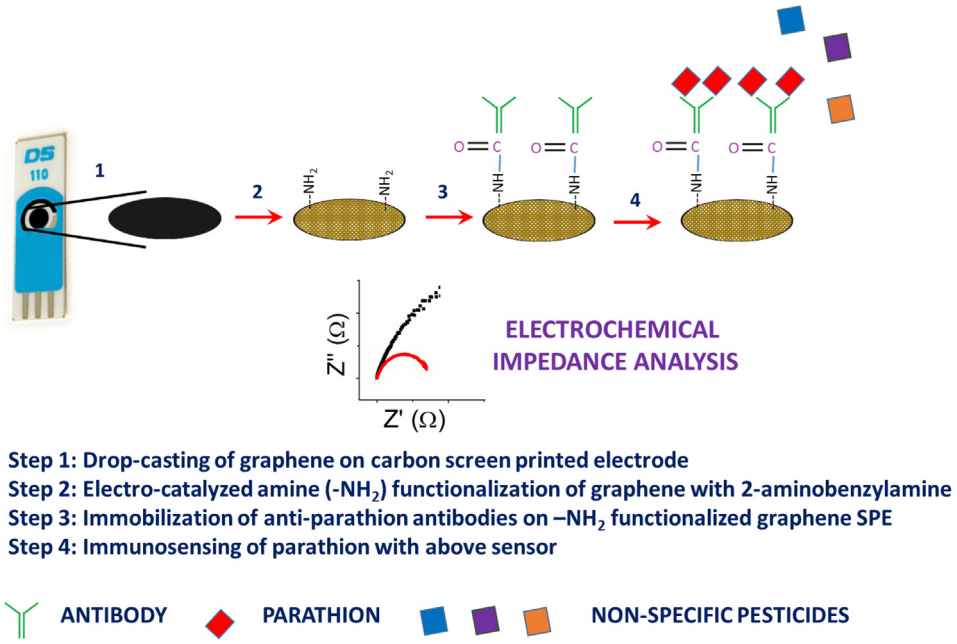

Fig. 1. Schematic of the graphene-based, screen-printed immunosensor for parathion.

(Bontidean et al., 2003; Cherian et al., 2003; Dzyadevych et al., 2003; Khosraviani et al., 1998; Mattiasson, 1997; Preininger and Wolfbeis, 1996). Electrochemical signaling based biosensors are immensely popular due to their sensitivity, selectivity, rapid response, and low-cost manufacturing (Takahashi et al., 2010; Goicolea et al., 2011). Various electrochemical biosensors have been proposed for the detection of OPs based on amperometry, potentiometry, voltammetry, and impedance analysis (Wu et al., 2013; Xue et al., 2013; Bhardwaj et al., 2015; Deep et al., 2015; Zhao et al., 2015; Chauhan et al., 2016). The electrochemical impedance spectroscopy (EIS) is an interesting class of the electrochemical techniques with an underlying capability of direct analyte detections via simple affinity complex formation. The EIS measurements involve the study of charge transfer resistance or capacitance at the electrode interface during probe-analyte interaction (Bourigua et al., 2010). The extension of EIS-based sensors to screen-printed electrodes is used to realize compact and cost-effective detection systems (Arora et al., 2011).

Recent developments in nanomaterials have made it possible to further improve the sensitivity levels of the pesticide biosensors (Rosi and Mirkin, 2005; Du et al., 2009; Aragay et al., 2012; Audrey et al., 2012; Kumar et al., 2012, 2015; Li et al., 2012; Bhardwaj et al., 2015; Deep et al., 2015). Several studies have established graphene as a material of choice for the construction of highly sensitive and stable biosensors for a variety of analytes (Suri et al., 2009; Shao et al., 2010; Yola et al., 2014a, 2014b, 2016; Tuteja et al., 2015; Atar et al., 2016). Graphene is characterized with ultra-high charge mobility, high surface to volume ratio, high Young's modulus, and excellent target binding properties, which together result in enhanced selectivity and sensitivity (Wang et al., 2010, 2013; Atar et al., 2015). Various types of graphene containing nanoconjugates have also been widely explored for the sensing of different analytes (Akyıldırım et al., 2015; Yola et al., 2015; Kotan et al., 2016;).

The use of graphene for the development of pesticide biosensors has been reported for organophosphates and carbamates (Gong et al., 2011, 2012; Liu et al., 2011a, 2011b; Wu et al., 2013). Most of these sensors exploited the use of graphene in a nanocomposite form with other materials (Gong et al., 2011, 2012; Liu et al., 2011a, 2011b; Wu et al., 2013) and the related measurements relied on the mechanism of inhibition of the bio-immobilized enzyme's activity. In some studies, the graphene nanostructures have also been used to design screen printed biosensors (SPE) (Ping et al., 2011; Song et al., 2013; Yang et al., 2013). Ping et al. (2011) proposed the assembly of a reduced graphene oxide/ionic-liquid doped SPE for the biosensing of glucose with the aid of physically immobilized glucose oxidase. As another example, a chitosan-reduced graphene oxide–nickel nanocomposite was deposited on an SPE to realize an enzyme-free detection of glucose (Yang et al., 2013). A silver nanoparticles/graphene modified SPE has also been proposed for the sensing of immunoglobulin E (Song et al., 2013). The SPE had to be conjugated with a specific aptamer (bio-recognition molecule) via streptavidin beads. Recently, a graphene modified SPE has also been proposed for the analysis of cathecholamine neurotransmitter (Apetrei et al., 2014). These authors attached the required enzymatic bioprobe on the graphene surface via glutaraldehyde mediated conjugation process.

In this research, we explored the viability of a convenient graphene-SPE platform for non-enzymatic label-free immunosensing of parathion. Unlike the approaches employed in earlier reports, the herein used graphene SPE does not need any mixing with other materials. The bioconjugation process has also been straightforwardly performed without the use of cross linkers or any other cumbersome chemistry. Briefly, the SPE was first modified with the dispersed graphene sheets. The electrode surface was then electrochemically treated with the 2-aminobenzyl amine so as to generate the active $–NH_2$ functional groups. The above functionalized electrodes were then simply incubated with the anti-parathion antibodies to realize the desired biosensor. As the antibody molecules were bound to the graphene-modified SPE through the F_c region based on this approach, this oriented immobilization allowed the biosensor to have free F_{ab} regions of the bio-recognizing probe. As such, enhanced detection sensitivity of this biosensor is expected to be achieved by activating all the possible available antibody probes in the system. A full scheme of this proposed strategy is illustrated in Fig. 1. The developed graphene SPE immunosensors has offered a highly sensitive and a specific electrochemical detection of parathion.