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Sensitive bi-enzymatic biosensor based on polyphenoloxidases–gold nanoparticles–chitosan hybrid film–graphene doped carbon paste electrode for carbamates detection

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ABSTRACT

A bi-enzymatic biosensor (LACC–TYR–AuNPs–CS/GPE) for carbamates was prepared in a single step by electrodeposition of a hybrid film onto a graphene doped carbon paste electrode (GPE). Graphene and the gold nanoparticles (AuNPs) were morphologically characterized by transmission electron microscopy, X-ray photoelectron spectroscopy, dynamic light scattering and laser Doppler velocimetry. The electrodeposited hybrid film was composed of laccase (LACC), tyrosinase (TYR) and AuNPs entrapped in a chitosan (CS) polymeric matrix. Experimental parameters, namely graphene redox state, AuNPs:CS ratio, enzymes concentration, pH and inhibition time were evaluated. LACC–TYR–AuNPs–CS/GPE exhibited an improved Michaelis–Menten kinetic constant (26.9 ± 0.5 M) when compared with LACC–AuNPs–CS/GPE (37.8 ± 0.2 M) and TYR–AuNPs–CS/GPE (52.3 ± 0.4 M). Using 4-aminophenol as substrate at pH 5.5, the device presented wide linear ranges, low detection limits ($1.68 \times 10^{-9} \pm 1.18 \times 10^{-10}$–$2.15 \times 10^{-7} \pm 3.41 \times 10^{-9}$ M), high accuracy, sensitivity ($1.13 \times 10^{6} \pm 8.11 \times 10^{4}$–$2.19 \times 10^{8} \pm 2.51 \times 10^{7}$ %inhibition M^{-1}), repeatability (1.2–5.8% RSD), reproducibility (3.2–6.5% RSD) and stability (ca. twenty days) to determine carbaryl, formetanate hydrochloride, propoxur and ziram in citrus fruits based on their inhibitory capacity on the polyphenoloxidases activity. Recoveries at two fortified levels ranged from 93.8 ± 0.3% (lemon) to 97.8 ± 0.3% (orange). Glucose, citric acid and ascorbic acid do not interfere significantly in the electroanalysis. The proposed electroanalytical procedure can be a promising tool for food safety control.

1. Introduction

Carbamates are one of the principal classes of pesticides that are being largely used to increase crop yield. However, their residues may pose serious environmental and health problems [1,2]. The adverse effects of several carbamates were reported, and they include renal, hepatic, neurological, reproductive, immune, and metabolic functions in both humans and animals [3,4]. Some of them are classed as endocrine disrupting chemicals [5] and regarded as priority pollutants by the United States Environmental Protection Agency [6].

Biosensor technology has been considered as a key tool for the implementation of the new European Union directives because of the negligible waste generation, minimization of use of hazardous substances, high sensitivity and selectivity, as well as, the in situ real-time monitoring capacity [7,8]. In this perspective, the biosensing of environmental pollutants, particularly agrochemicals, using enzymes as biorecognition element has increased pronouncedly in the last years [1,9–14]. Still, many of these devices need to improve their performance because of the low maximum residue limits (MRLs) established worldwide for pesticides [15,16]. Considerable positive synergistic effects on the current signal can be attained by combining several enzymes [17–21]. Enzyme selection and their sources have a major influence on the biosensor sensitivity [19,22]. The few studies dedicated to bi-enzymatic biosensors [17–21,23–25] reported in the last ten years are summarized in Table 1S (Supplementary material). As far as the authors know, there is no publication related to the application of bi-enzymatic biosensors for the quantification of pesticides in food commodities or in other real samples. Moreover, there is a general lack of validated biosensor-based procedures for analysis of food samples [1,12,13,26].

The main drawback of the application of enzymatic biosensors to complex matrices is the susceptibility of the transducer to surface passivation. Furthermore, enzymatic products may undergo partial

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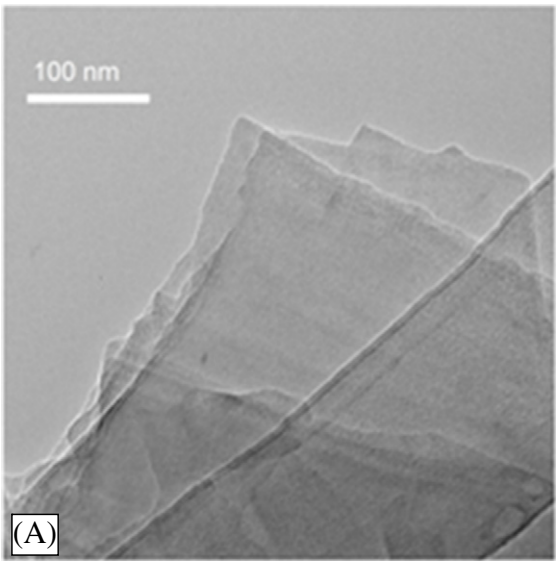
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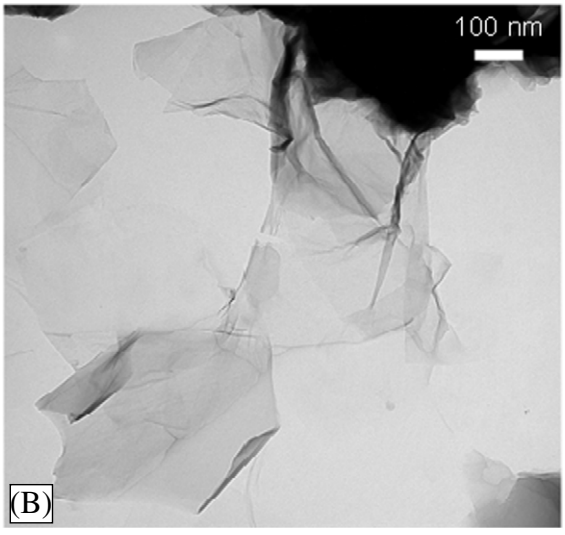
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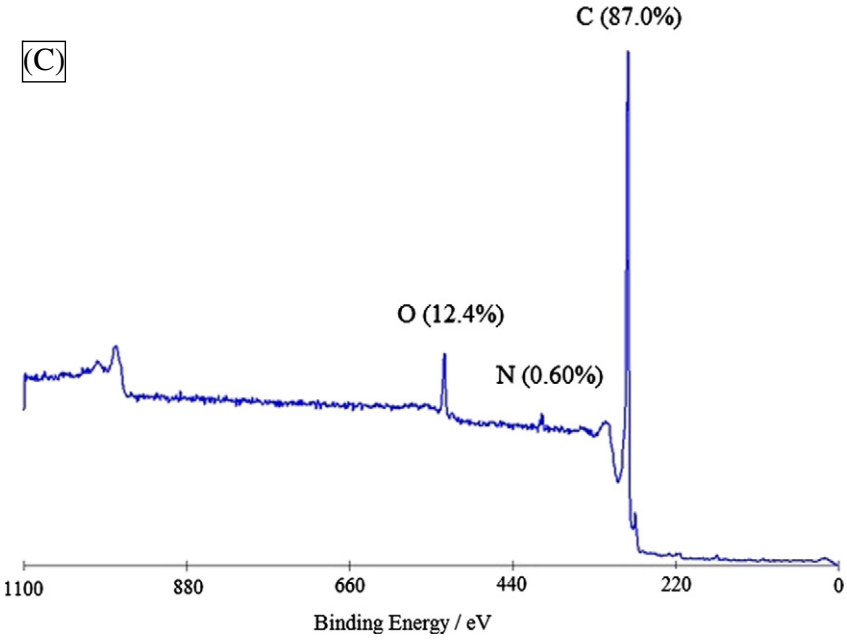
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