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# Electrochemical biosensors for biocontaminant detection consisting of carbon nanotubes, platinum nanoparticles, dendrimers, and enzymes

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ABSTRACT

The presented approach provides the advanced development of effective, rapid, and versatile electrochemical sensors for a small amount of analytes on potential, cheap, and disposable printed chips. The electrocatalytic activity of this biosensor revealed the feasible detection of hydrogen peroxide at low potential (~0.09 V) and the detection of a biocontaminant inhibitor (organophosphorus pesticide) in a wide range of concentrations. This efficiency comes from the chemical immobilization of catalysts (Pt nanoparticles) and electron transfer-enlarging materials (carbon nanotubes) on an electrode. Especially, dendrimers raise the stable conjugation of enzymes (acetylcholinesterase/choline oxidase/peroxidase) as well as nanoparticles and carbon nanotubes on an electrode.



The utilization of metal-based nanoparticles as an immobilization site for enzymes has been developed for biosensing approaches [1,2]. Platinum nanoparticles (PtNPs) can catalyze excellently an electrochemical reaction on oxidation of $H_2O_2$, which is generated by the enzymatic reaction [1,3]. Meanwhile, multiwalled carbon nanotubes (MWCNTs) can be employed to modify the working electrode for the enlargement of the electroactive surface area, which leads to an increase in the electron transfer properties of the biosensing system [4]. Electrodes modified by hybrids of carbon nanotubes (CNTs) and metal nanoparticles have been developed for use as fuel-cell catalysts and biosensors [5–8]. A Pt–CNT–glucose biosensor was developed using the incorporation of glucose oxidase (GOx) on a Pt–CNT electrode [6]. Even though this biosensor revealed a high sensitivity toward glucose oxidation, its stability after storage for a few days decreased owing to the release of GOx that is not entrapped within the $Pt_{nano}/SiO_2$ composite matrix as a binder.

In a previous work, electrodes modified by hybrids of CNTs and PtNPs protected by dendrimers were developed for electrochemical methanol oxidation [8]. In the present work, chemically stable electrochemical biosensors consisting of an electrode loading MWCNTs and PtNPs were developed by the mediation of poly(amido amine) dendrimer (DEN) as a binder and the immobilization of enzymes on them as shown in Scheme 1. The goal of this study was to test the feasibility of this electrode. Thus, the detection of an organophosphorus pesticide through the inhibition of the acetylcholinesterase (AChE) enzyme reaction was investigated by cyclic voltammetry (CV) to judge the availability of the present sensing system. In the sensors, carbon nanotubes are expected to enlarge the surface area of the working electrode and promote the electron-transfer properties. PtNPs can also intensify the electron-transfer ability in addition to catalytic performance, and dendrimers play a role in the uptake of the redox agent as well as the binder functionality. Thus the CNT/DEN(PtNPs)-loaded electrode possesses high durability and reactivity [8].

Experimental details and tables of results are provided in the Supplementary material. For the electrochemical detection of diazinon oxon (DZN), CNT/DEN(PtNPs) hybrids were fabricated onto a disposable electrochemical printed (DEP) chip with a screen-printed circular glassy carbon (SPCGC) working electrode. The characterization of CNT/DEN(PtNPs) hybrids was reported previously [9]. In addition, it has been reported that the CNT/DEN(PtNPs)-loaded SPCGC electrode possessed high stability, since DEN(PtNPs) and CNT/DEN(PtNPs) still retained their physical and chemical immobilization on the DEP chips through binders/stabilizers even after 20 cycles of the CV scan [8]. Therefore DEN plays an important role as a stabilizer and binder in CNT/DEN(PtNPs)-loaded SPCGC electrodes [8,9]. Successively, enzymes were loaded

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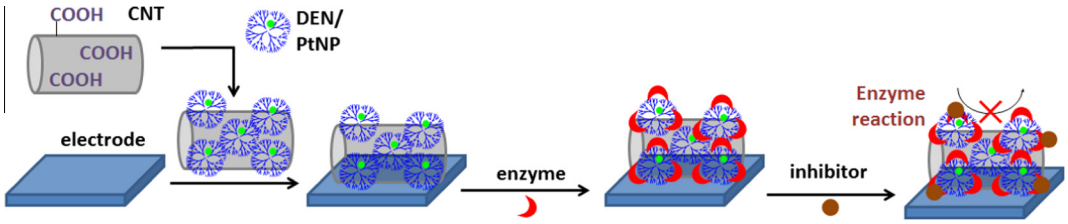


**Scheme 1.** Schematic illustration of preparation of biosensor, loading multiwalled carbon nanotubes, platinum nanoparticles, and enzymes through dendrimer binders.

on the electrode, and the bioactivity of the as-prepared biosensor was investigated by CV. It is well known that AChE plays an important role in cholinergic transmission as a catalyst for the rapid hydrolysis of the neurotransmitter acetylcholine into acetate and choline. The choline is subsequently converted by choline oxidase (ChO), producing hydrogen peroxide in the presence of oxygen [4,5].

The inhibition of AChE activity by DZN can be determined by measuring the redox current of $H_2O_2$, which is correlated with the concentration of choline produced by AChE. Enzymes were used for the detection of organophosphorus compound in this work, but the activity of the enzymes is affected by the chemical environment (e.g., pH, temperature, ionic strength, and so on). Therefore, for biosensors based on an enzyme system, the sensor activity may also depend on such environments. It has been reported that the optimum pH for ChO enzyme activity is between 7.0 and 8.0 and that for AChE is between 8.0 and 9.0 [10]. It was also found that choline will lose its activity at pH < 6.0, and the hydrolysis of organophosphorus compound was observed at pH > 8.0 [4]. For the case of choline electrochemical biosensors, the choline sensing was carried out in phosphate buffer solution at pH 7.5 [11]. Thus, in view of these reports, electrochemical detection of DZN was carried out in a phosphate buffer solution at pH 7.4 to maintain both the stability and the activity of the AChE, ChO, and peroxidase (POD) enzymes. On the other hand, the inhibition of AChE activity by organophosphorus compound is an irreversible process; when the enzyme is exposed on the organophosphorus compound, the enzyme is inactivated and therefore the sensor can be reused only after an appropriate enzyme reactivation. Thus, biosensors that are low cost and disposable are highly desirable in this application [12,13].

The cyclic voltammograms of an enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode in a 10 mM phosphate buffer (pH 7.4) solution of acetylcholine chloride showed a well-defined anodic oxidation peak at the potential around 0.09 V with an anodic peak current density of 0.12 mA/cm$^2$, as seen in Fig. 1, although such peaks were not observed on the electrode without acetylcholine chloride (Fig. 1, control) and without loaded enzymes (Supplementary Fig. S1). This peak might be attributed to the oxidation of $H_2O_2$ generated from the enzymatic reaction of AChE and ChO [1,2,10,14]. It might be noticed that the peak potential of $H_2O_2$ oxidation by the present electrode is lower than by an AChE/ChO/CNT electrode (0.50 V) [3]. This indicates that the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode possesses an excellent electrocatalytic activity toward the oxidation of $H_2O_2$ at low potential, and it might be attributed to the presence of DEN(PtNPs) on this biosensor. The low oxidation potentials reveal an advantage of this electrode, because only low potential is needed for the detection and there is no interference from the other electroactive species in the sample derived from the high applied potential. Meanwhile, a reduction peak around −0.5 V was also observed. This peak is attributed to the reduction of the generated $H_2O_2$ by POD. Hence, the inhibition of enzyme activity of AChE by DZN can be determined by the decrease in the oxidation or reduction current at 0.09 and −0.5 V, respectively.

With addition of DZN to the solution of acetylcholine chloride, an anodic oxidation peak around 0.09 V shifted to lower potential and decreased in the current density. It can be suggested that the obtained decrease in the anodic current is due to the inhibition of the AChE enzyme activity by DZN, leading to a diminution in the concentration of choline and in the amount of $H_2O_2$ generated from the enzymatic reaction.

The obtained currents of the anodic oxidation peak were utilized to evaluate the enzyme activity, $I_o/I_x$, and the inhibition value, $(I_o - I_x)/I_o$, where $I_o$ and $I_x$ are taken from current densities in solutions of acetylcholine chloride without and with DZN. The calculated values are listed in Supplementary Table S1 and plotted in Fig. 2a, where the regression values of the linear relations were 0.78 and 0.97 for $Na_2PtCl_6$ to the amino-terminal group of the dendrimer (*M:D*) = 0.2:1 and 0.4:1, respectively. It was found that the enzyme activity decreased and, therefore, the inhibition percentage increased with increasing DZN concentration. A linear relationship between the inhibition percentage and the logarithmic DZN concentration covered a wider DZN concentration range below 10 ppm than in the previous report [15]. It can be noted that the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode reveals a significant detection performance for DZN based on the inhibition of enzyme reaction at low potential around 0.09 V.

Platinum is very active in the electro-oxidation of $H_2O_2$, and a PtNP electrode oxidizes at a lower potential than a Pt bulk electrode. It was observed in Fig. 2a that the detection of $H_2O_2$ by the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode at *M:D* = 0.4 was higher than at *M:D* = 0.2. The percentage inhibition at various *M:D* ratios toward the detection of $H_2O_2$ in the presence of 0.1 ppm DZN is shown in Fig. 2b (numerical values are listed in Supplementary Table S1). It was observed that the percentage inhibition of AChE activity significantly increased from 15% at

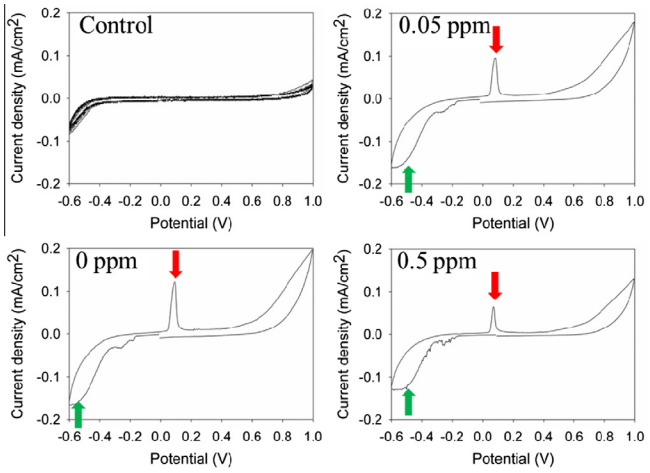


**Fig.1.** Cyclic voltammograms of enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrodes in a 10 mM phosphate buffer solution (pH 7.4) of acetylcholine chloride. Numerical values denote the concentration of DZN. Control experiment was carried out in 10 mM phosphate-buffered saline (pH 7.4) without acetylcholine chloride.

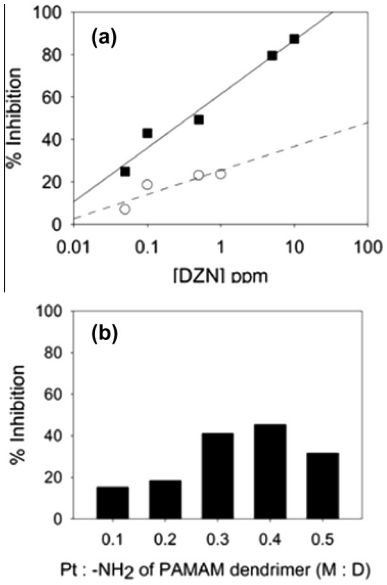


**Fig.2.** Plots of % inhibition of acetylcholinesterase enzyme reaction (a) by diazinon oxon using the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode at *M:D* = 0.2:1 (○) and 0.4:1 (■) and (b) in the presence of 0.1 ppm diazinon oxon using the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode at various *M:D* ratios.

*M:D* = 0.1:1 to 43% at *M:D* = 0.4:1, although the percentage inhibition decreased at *M:D* = 0.5:1. The increase in the percentage inhibition of AChE activity against *M:D* might be due to the promotion of the oxidation of $H_2O_2$, which was performed by PtNPs on the electrode. Thus, it could be suggested that the percentage inhibition depends on the amount of PtNPs loaded on the electrode, similar to a previous report [16]. It has been reported that the electrocatalytic activity of the catalyst is influenced by the composition, the content, and the activity efficiency of the electrocatalyst, leading to the required current [17].

In conclusion, the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode, which was fabricated using the immobilization of three enzymes (AChE, ChO, and POD) on a CNT/DEN(PtNPs)-loaded SPCGC electrode, showed a desirable electrocatalytic activity on the enzyme reaction toward the oxidation and reduction of $H_2O_2$ at low potential around 0.09 and −0.5 V, respectively, and the significant detection of a biocontaminant (DZN) based on the inhibition of enzyme reaction in a wide range of DZN concentrations. In addition, the sensitivity of the detection of $H_2O_2$ by the enzyme/CNT/DEN(PtNPs)-loaded SPCGC electrode depends on the amount of PtNPs loaded on the electrode. This indicates the remarkable sensitivity and the selective detection ability of the present electrode for any kind of target molecule, if an adequate molecular recognition or reaction system is loaded on the electrode. Above all, dendrimers can play a role in not only the binding of CNTs and PtNPs on electrodes but also the stable conjugation of enzymes. Moreover, the DEP chips are adequate for a small amount of analytes and rapid testing. Therefore, the present approach provides the advanced development of an effective and versatile electrochemical sensing system for wide application as biomedical, food, chemical, and environmental sensors.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ab.2013.09.004.

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