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A highly selective and sensitive nanosensor for the detection of glyphosate

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ABSTRACT

A turn-off fluorescence sensor synthesized by combining copper (II) oxide and multiwall carbon nanotubes (MWCNTs) were used for measuring glyphosate based on the inhibiting the catalytic activity of the CuO/MWCNTs. This sensor was synthesized by precipitating copper ions onto the acidic MWCNTs under basic conditions; the resulting material was characterized by the transmission electron microscopy, X-ray photoelectron spectroscopy, and Fourier transform infrared spectroscopy to confirm its structure. The CuO/MWCNTs nanomaterial was found to exhibit high peroxidase-like catalytic activity toward the reduction of H_2O_2 to H_2O and the oxidation of Amplex Red to resorufin, with a corresponding color change from pink to red and the fluorescence enhancement. However, this activity was inhibited and the fluorescence diminished when glyphosate was added to the system. Using this strategy, we applied this sensor to detect glyphosate. The results indicated that this sensor is not only highly sensitive, with a detection limit of 0.67 ppb and a linear range from 0.002 to 0.01 ppm, but also exhibits good selectivity for glyphosate. When this sensor was assessed for detecting glyphosate in real water samples, recoveries of 96–107% were attained. This proposed material and method are a promising approach for rapid screening of glyphosate.

1. Introduction

Many organophosphate compounds can irreversibly inactivate acetylcholinesterase (AChE), an essential enzyme for nerve function in organisms, and cause different physiological reactions even death. Thus, these compounds could be used as drugs [1], herbicides, chelating agents, and chemical warfare agents. Glyphosate (N-(phosphonomethyl)glycine), an organophosphate pesticide commonly used worldwide, is one of the first herbicides to be used to against crops that have been genetically modified to increase their tolerance. Commercial glyphosate-based formulations most commonly comprise concentrates containing approximately 1–41% glyphosate; these products are marketed for agricultural use [2]. Glyphosate residues have the potential to contaminate surface waters and to enter the food chain because of its aquatic use patterns and good water solubility. The median half-life of glyphosate in water varies from a few to 91 days. The US Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of glyphosate of 0.7 ppm in drinking water. Therefore, monitoring of glyphosate in crops, fruits, vegetables, and drinking water has become increasingly important. The difficulty in finding simple detection methods stems from the fact that glyphosate is highly polar and highly soluble in water while also exhibiting low volatility, and lacking a chromophore or fluorophore in its molecular structure.

In the traditional analytical method, the quantitative determination of glyphosate has generally relied on chromatography methods after the pretreatment steps of real sample such as liquid-liquid extraction or solid-phase extraction [3,4]. In addition, several others methods have been developed for the detection of glyphosate including HPLC [5], capillary electrophoresis [6,7], mass spectrometry [8,9], fluorescence [10], gas chromatography [3] and even enzyme-linked immune sorbent assay [11].

Recently, nanomaterial based sensors have emerged for detecting numerous small molecules, including organophosphate pesticides. Most of these methods can not only analyze samples *in situ*, but also avoid many of the disadvantages associated with other methods, such as complicated sample preparation procedures, laborious analytical methods, and complicated and expensive instrumentation. Lipoic acid (LA)-capped gold nanoparticles (AuNPs) have been used to detect organophosphate (OP) nerve agents and pesticide because the degree of AuNPs'

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aggregation is strictly dependent on the concentration of thiocholine, which is a product of AChE. In the presence of an OP, the capped AuNPs do not aggregate, because the catalytic activity of AChE is inhibited, resulting in decreased the thiocholine formation [12]. Rhodamine B (RB)-functionalized AuNPs (RB-AuNPs) have also been used to detect pesticides via a similar strategy. Specifically, the electrostatic interaction between RB and AuNPs leads to the quenching of RB's fluorescence. Because the thiocholine produced from AChE substitutes RB on the surface of AuNPs, the fluorescence of RB is recovered and the color of the solution changes from red to purple [13]. Au nanocluster stabilized by DNA/protein have also been used to detect OPs by the decrease of fluorescence intensity caused by hydrogen peroxide generated as a byproduct of AChE hydrolysis [14]. Some enzyme-free nanosensors have also been developed. For example, a turn-on fluorescence assay based on the fluorescence resonance energy transfer (FRET) between negatively charged CdTe quantum dots and positively charged AuNPs was used to measure glyphosate [15]. In other studies, a copper doped poly(vinyl) alcohol nanofiber changed the color of solution from blue to yellow when the sample contains dithiocarbamic acid [16]. Most of these previously reported sensors are highly depended on the activity of AChE. However, the enzyme activity is easily influenced by the pH, ionic strength, temperature and other chemical conditions.

In this study, we try to design and synthesize a high sensitive, selective and enzyme-free sensor for the detection of glyphosate in water sample; this sensor is based on the inhibition of peroxidase-like activities of a nanomaterial. We expect that this enzyme-free sensor could be applied to the detection of environmental samples.

2. Experimental

2.1. Reagents and chemicals

MWCNTs (Outer diameter <8 nm, length 10–30 μm, carbon purity $>95\%$), NaOH, H_2SO_4 (98%), and other pesticides were from Sigma-Aldrich. Glyphosate was purchased from Fluka. Tris, boric acid were purchased from J.T Baker. Amplex Red (AR) was obtained from Invitrogen. Hydrogen peroxide was from Acros Organics. Water samples were taken from the Taitung Flowing Lake in the Taitung Forest Park and the tap water of school (Laboratory). All chemical reagents were of analytical reagent grade and were used without further purification.

2.2. Instrumentation

The morphologies of the samples were analyzed with transmission electron microscope (Tecnai G2 F20 S-TWIN, FEI Company, USA). X-ray photoelectron spectroscopy (XPS) spectra were carried out with a K-Alpha diffractometer (Thermo scientific, USA). The FT-IR spectrum was obtained from a Nicolet 5700 (USA) IR spectrometer in the range of 400–4000 cm^{-1}. Versatile disc fluorescence/absorption spectrometer spectra were obtained from Infinite® M200 Multimode microplate reader (Tecan Instruments, USA).

2.3. Preparation of CuO/MWCNTs nanomaterial

The pristine MWCNTs (0.5 g) was first oxidized by refluxing in 98% sulfuric acid and 70% nitric acid (3:1 v/v) mixture for 24 h at 80 °C in double-neck flask. Then, the products were washed with deionized water by repeated centrifugation at 12,000 rpm for 10 min until the pH value was adjusted to 7. The product was dried for 48 h at 80 °C. The CuO was deposited on the MWCNTs by adding sodium hydroxide in the cooper nitrate solution. The catalytic activity of CuO/MWCNTs nanomaterial is dependent on the concentration of $Cu(NO_3)_2$, NaOH and the amount of MWCNTs. Various concentrations of these reagents were used to synthesis the catalyst and the optimum experiment conditions were chosen for the maximum activity of the catalyst. The mixture of copper ion and MWCNTs were heated to 80 °C for 10 min and difference concentrations of NaOH were introduced into the above solution reacting for another 60 min at 80 °C. After the reaction was complete, the products were collected by centrifugation and washed with deionized water several times until the pH reach 7. Finally, the resulting precipitates were dispersed in deionized water for characterization and further used.

2.4. Determination of glyphosate

A stock solution of glyphosate (100 ppm) was prepared in water and various concentrations of glyphosate were obtained by serial dilution of the stock solution. For the detection of glyphosate, first, the CuO/MWCNTs were diluted 20-fold with deionized water, then pipetted 100 μL into the 1.5 mL vials and treated by centrifugation at 14,000 rpm for 10 min to removed 80 μL of the supernatants. Then the other 80 μL glyphosate solutions with different concentrations were added into above solution and the mixed solutions are remained shaking for 30 min. To investigate the activity of the peroxidase-like catalyst of CuO/MWCNTs, 120 μL of deionized water, 20 μL of the catalyst suspension, 20 μL of AR (1 mM), 20 μL of 1 mM H_2O_2 and 20 μL of 100 mM Tris borate buffer (pH 7.12) were mixed sequentially. The mixture was incubated for 120 min and the resulting solution was treated by centrifugation at 14,000 rpm for 10 min. Finally, the supernatants were subjected to fluorescence measurements with excitation and emission wavelengths of 550 and 584 nm, respectively.

2.5. Real samples sensor

Water samples were prepared following a previous method after a simple pre-treatment. Briefly, the water samples were filtered with 0.22 mm filter and centrifuged at 14,000 rpm for 10 min by ultrafiltration (MWCO = 3 K) an the following process were the same with the above experiment.

3. Results and discussion

3.1. Synthesis of CuO/MWCNTs

Copper(II) oxide nanoparticle/multiwall carbon nanotubes (CuO/MWCNTs) were prepared by precipitating copper nitrate by the addition of aqueous NaOH solution; this procedure was conducted at 80 °C for 1 h. The resulting nanomaterials exhibited peroxidase-like activities that catalyzed the reduction of H_2O_2 to H_2O and the oxidation of colorless Amplex red (AR) reagent to red resorufin via electron transfer process. The intensity of fluorescence from resorufin under excitation at 550 nm and emission at 584 nm was measured as the catalytic activity of these nanomaterials.

The catalytic activity of CuO/MWCNTs nanomaterial was observed to depend on the concentrations of $Cu(NO_3)_2$, NaOH, and MWCNTs used in the synthesis. The experimental results show that the catalytic activity of the CuO/MWCNTs increased with the $Cu(NO_3)_2$ concentration from 100 to 600 mM; at higher concentration, the catalytic activity slightly decreased as the $Cu(NO_3)_2$ concentration increased (Fig. S1A). We believe that the carboxyl group in the oxidized MWCNTs limited the amount of CuO formed. The effect of NaOH concentration was investigated in the range from 1.0×10^{-3} to 10 M (Fig. S1B). As the concentration increased