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A facile and sensitive detection of organophosphorus chemicals by rapid aggregation of gold nanoparticles using organic compounds

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ABSTRACT

Organophosphorus (OP) chemicals are highly effective insecticides and germicides, and are the most widely used in agriculture. Unfortunately, OP compounds are some of the most toxic substances to humans, even at very low doses. Because detecting OP residues in agricultural products is essential, simple, sensitive, and particularly rapid on-site detection methods are required. Gold nanoparticles (AuNPs) have been used as signal-enhancing detection probes in the field of biosensors due to their size-dependent optical properties. When imidazole was added to AuNPs mixed with OP compounds, the AuNPs was aggregated and their color changed to purple. This caused the appearance of a new peak at 660–670 nm, which could be measured within approximately 30 s. Therefore, this method allows the detection of OP compounds, including diazinon, iprobenfos, and edifenphos, on-site at part-per-billion (ppb) concentrations, and also affords a straightforward method. Furthermore, the method was successfully applied in the determination of OP compound in a real sample (river water) with satisfactory results.

1. Introduction

In agriculture, farmers use numerous agrichemicals to protect crops and seeds before and after harvesting. Agrichemicals is a broad term used that encompasses the organic toxic compounds used to control insects, bacteria, weeds, nematodes, rodents and other pests (Sassolas et al., 2012). Among these, organophosphorus (OP) chemicals are used worldwide, and so large amounts of OP residue could cause contamination by accumulating in the environment including the air, soil, water, and agricultural products. This could eventually lead to serious health concerns for humans even after exposure to only very low concentrations (Zhang et al., 2014; Yang et al., 1995). The high toxicity of OP compounds is caused by their ability to inhibit acetylcholinesterase (AChE), which is an important enzyme that hydrolyzes acetylcholine. Inhibiting AChE activity allows acetylcholine to accumulate in cholinergic clefts, which overstimulates both the peripheral and central cholinergic nervous systems, and has fatal consequences (Yadav et al., 2012). The symptoms of OP poisoning include headache, dizziness, salivation, lacrimation, sweating, vomiting, diarrhea, abrupt tremor, lung edema, coma, and even death from respiratory or cardiac failure (Meng et al., 2013). The development of a sensitive and inexpensive diagnosis tool for environmental and biological monitoring is a current research area to facilitate the removal of excess OP compounds, to prevent disease, and protect the environment. Moreover, it is essential to develop a method that allows the on-site and real-time detection of OP residues rapidly and easily.

Several methods to detect OP compounds have been developed over the past decade. For example, liquid or gas chromatography coupled to mass spectrometry, electrochemical analysis, fluorescent bioprobes, and enzyme-linked immunosorbent assays (ELISAs) have been used to detect OP compounds (Mehrvar and Abdi, 2004; Rao et al., 2002; Trojanowicz, 2009; Shi et al., 2006; Jeanty and Marty, 1998; Chen et al., 2009; Meng et al., 2013). These methods have high selectivity, adequate sensitivity, and reliability; however, most are also associated with disadvantages, such as high costs, the need for sophisticated instruments, the requirement for highly qualified and trained operators possessing master professional skills, tedious sample pretreatment, and expensive biomolecular reagents. Therefore, these methods are not suitable for on-site detection in most settings (Zhang et al., 2014; Yi et al., 2013).

AuNPs-based colorimetric assays are effective approaches for quantifying many types of analytes without the need for complex equipment because molecular alterations can cause color changes in the AuNPs (Jiao et al., 2014; Sener et al., 2014; Feng et al., 2013; Wang et al., 2013; Zhang et al., 2010; Li et al., 2010; Huang et al., 2005; Liu et al., 2010; Wang et al., 2006). The color of AuNPs is

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size- and shape-dependent and highly sensitive to aggregation states. It can be easily measured by a UV/vis spectrophotometer (Feng et al., 2013). In general, well-dispersed AuNPs are colored red, whereas aggregated AuNPs are purple- or blue-colored. The AuNPs color change can be measured on the basis of the UV/vis absorption spectrum via a shift in the maximum peak and/or the appearence of a second peak in the visible region (Jiao et al., 2014; Sener et al., 2014; Feng et al., 2013; Wang et al., 2013; Zhang et al., 2010; Li et al., 2010; Huang et al., 2005; Liu et al., 2010; Wang et al., 2006; Chegel et al., 2012).

Imidazole has been reported as an alkaloid organic compound in relation to conjugation potential. Due to the coexist of position 1 protonated nitrogen of pK_a 14.0 and position 3 nitrogen of pK_a 6.5, imidazole has an amphoteric property with dual-function as both an acid and a base (Walba and Isensee, 1961; Holze, 1993). The property, through the formation of weak complexs between *N*-ligands and *O*-ligands (Lorenzo et al., 1992), enables the imidazole to serve as precursors for the adsorption of other molecules onto metal surfaces (Xue et al., 1988; Wang et al., 2002; Cao et al., 2003). Furthermore, the strong affinity between imidazole and AuNPs induces the aggregation of the AuNPs, which results in a shift in the surface plasmon resonance into the near-infrared (NIR) wavelengths (Glauco et al., 2006). Therefore, the imidazole can be used as an aggregation promotor of AuNPs.

For the optical detection of OP compounds, we herein developed a simple and rapid biosensing method based on the aggregation of AuNPs in a two-step process. The proposed sensing strategy for the optical assay to detect OP compounds is shown in Scheme 1. Different concentrations of diazinon were mixed with the AuNPs, followed by addition of imidazole. Finally, the color of the AuNP suspension was immediately changed from red to dark blue (or purple) because of the aggregation of the AuNPs. We selected diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimiinyl)phosphorothioate] as the model OP, because it is a moderately persistent pesticide that is widly used in agriculture (Banaee et al., 2011). To verify this novel bioassay method, two additional OP compounds, iprobenfos and edifenphos, were tested at the concentration range of 0–10 ppm. This strategy for OP detection was performed without complex modification processes, enzyme immobilization, and/or the addition of salt. The assay results could be measured as soon as the reaction finished, which was within a few seconds.

2. Experimental

2.1. Materials

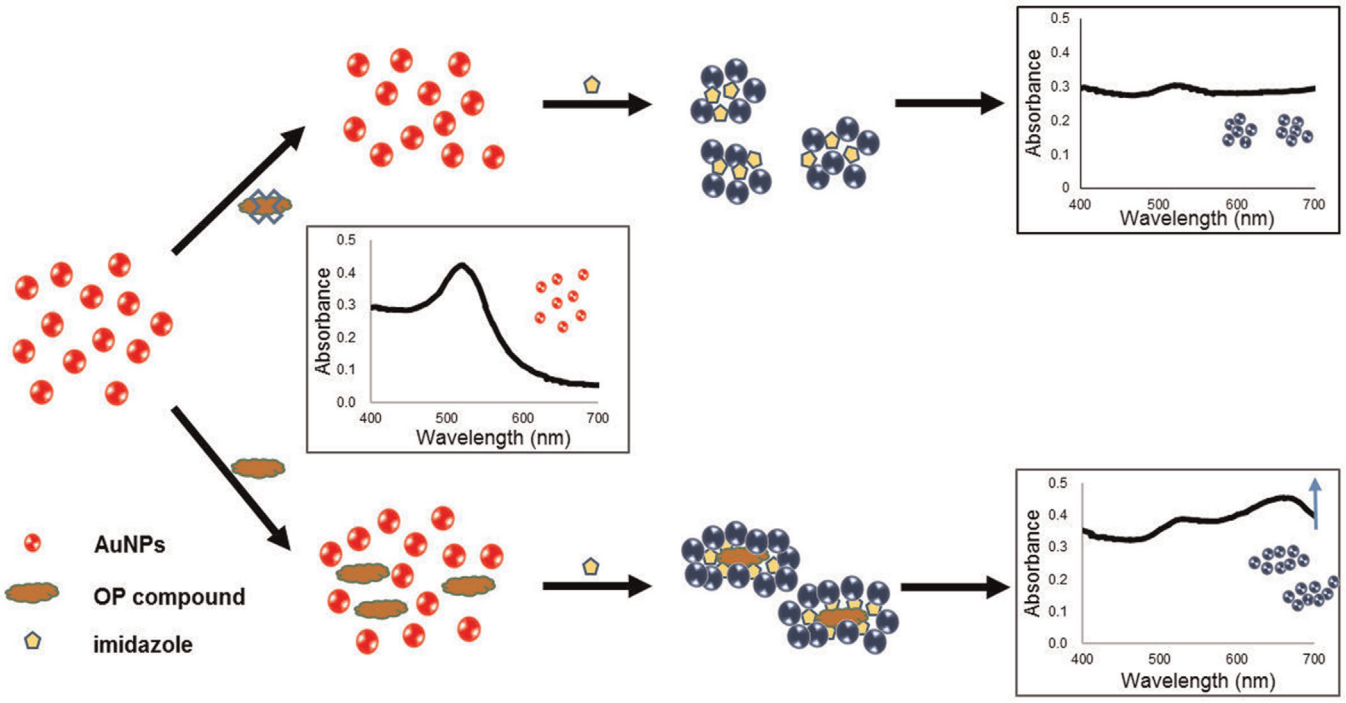
Gold(III) chloride hydrate ($HAuCl_4$), diazinon, iprobenfos, edifenphos, and phosphate-buffered saline (PBS, pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Trisodium citrate dehydrate, imidazole were manufactured by Bio-Basic (Ontario, Canada). Methanol was obtained from Merck chemicals (Darmstadt, Germany). Milli-Q grade distilled (DI) water (18.2 MΩ cm, Millipore, Billerica, MA) was used in all experiments.

2.2. Synthesis of citrate-stabilized AuNPs (Cit-AuNPs)

Dispersed AuNPs were prepared using a citrate reduction of $HAuCl_4$ (Kim et al., 2011). One hundred milliliters of DI water containing 1 mM $HAuCl_4$ was refluxed during stirring. When the $HAuCl_4$ solution started to boil, 10 mL of 38.8 mM trisodium citrate dihydrate was added, and the reaction was continued for 15 min to reduce the salt content. The color of the solution changed immediately to dark blue and then to dark red, indicating the formation of dispersed Cit-AuNPs. After a 15-min reaction, the solution was cooled while being stirred at room temperature, and was then stored at 4 °C. The synthesis of Cit-AuNPs was confirmed by recording UV/vis spectrum data and analysis using FE-TEM (Tecnai G2 F30 S-Twin, FEI, Hillsboro, OR).

2.3. Preparation of imidazole and OP solution

Imidazole was prepared by diluting the concentrated stocks in PBS solution (pH 7.4). The various concentrations of OP compounds were prepared by dissolving 10 μL of OP in 10 mL of 10%



Scheme 1. Schematic illustration of optical assay used to detect OP chemicals.