Sensors and Actuators B 210 (2015) 803–810

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A novel test strip for organophosphorus detection

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ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form
24 November 2014
Accepted 10 December 2014
Available online 19 January 2015

Keywords:
Test strip
Organophosphorus
Gold nanoparticles
Hydrogel

ABSTRACT

Herein we present a novel sandwich structure test strip for organophosphorus (OP) assessment, which is applicable and portable for field application. The middle layer of the strip is made of rhodamine B-covered gold nanoparticles (RB–Au NPs) entrapped in agarose and its coated layer is made of hydrogel (Poly ethylene glycol diacrylate) used as protectors for the center agarose layer. The OP assessment is based on the measurement of the concentration changing of thiocholine (generated from acetylthiocholine catalyzed by acetylcholinesterase in tested solution) when OP exist. The strip was further validated by various pesticide samples. Its detection limit was demonstrated to meet the maximum residue limits reported in the European Union pesticides database. Our strip also showed its good sensitivity and high reliability on testing river water samples, which suggested its great potential in environmental analysis.

1. Introduction

Organophosphorus compounds (OPs) [1,2], including phosphate ester compounds and their derivatives, are world widely used pesticides in agriculture today. These compounds exhibit severe toxicity on human nervous system by inhibiting acetylcholinesterase (AChE) activity [3,4], which leads to fatal consequences [5–7] as well as increases the risk of cancer [8]. With the increasing insecticide/herbicide resistance of the pests/weeds, more pesticides were developed and larger dosages were used, causing pesticide residue problems world wide. Moreover, some OPs were warfare agents used previously in the world war due to their toxicity. They are also potential weapons of terrorists to threaten public security [9]. At present, OP production and usage are strictly controlled by most countries, and accurate and frequent OP detection is essential for public safety and environmental safety.

In the past decades, many methods were developed for OP analysis, such as liquid/gas chromatography–mass spectrometry (LC–MS/GC–MS) [10,11], electrochemical analysis [12] and enzyme-linked immunosorbent assays (ELISAs) [13], etc. However, these conventional methods need either expensive instrumentations, complicated pretreatment of samples or skilled personnel for operation, which are not suitable for onsite analysis or large scale deployment for general public surveillance in most cases.

Recently, many relatively fast, cheap and convenient OP detection methods were developed, such as different kinds of test strips or kits [14,15]. However, there are still various limitations accompanying these products. For example, gold nanoparticles (Au NPs) indicator based OP-antibody chromatography test strips is limited by its antibody specificity, so that it was not feasible for complex pesticides analyses. AChE inhibition based test strip is suitable for complex OP conditions, but low sensitivity is its major drawback. So far, it is still in great need to develop OP detection approaches with good sensitivity and low cost for general applications.

Many polymers, such as hydrogel and agarose, are able to provide various platforms with microenvironments or microstructures for substance, such as cells [16], biomolecules [17,18], drugs [19], to perform specific chemical reactions or physical interactions, due to their special surface property, stability, flexibility, water-absorbability, nontoxicity, and non-immunogenicity, etc. Therefore, many hydrogels were applied in purification of biomolecules [20], drug-delivery systems [21,22], tissue or organ simulations [23], cartilage reparations [24,25], longtime storage of stem cells [26] and cell scaffolds for light controlled therapy [27], and so on.

Nanoparticles with specific optical properties have received much scientific and technical interest due to their unique and indicative function [28]. These materials with specific nanostructures can produce optical signal including fluorescence or color

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http://dx.doi.org/10.1016/j.snb.2014.12.048
0925-4005/

changes by assemblies and aggregations [29,30]. Au NPs are one of the most widely used nanoparticles with applications in nano-gold labeling technique [31], ELISA [32], immunoblotting [33], biosensor [34–36], gene-chip [37,38] and heavy metal rapid detections [39], etc. The aggregation states of Au NPs in solution can be recognized by color change because various sizes of aggregation have different light absorption [40]. This characteristic has been used as a colorimetric method for various onsite analyses, because the detection results can be checked by naked eye, without the need for advanced instruments. Recently, a highly sensitive, dual-readout (colorimetric and fluorometric) rhodamine-B (RB) functional Au NP-based assay for OP was reported [41]. This method has offered a better feasibility for real-time detection in public service. However, concerning about portability and usability for practical application, its operations based on solution is still a drawback.

Herein we combined the advantages of RB-functional Au NPs, agarose and hydrogel to develop a novel test strip for OP assessment which can be checked by naked eye. The agarose containing Au NPs was encapsulated in hydrogel and fixed on the surface of a test strip. Glyphosate, malathion, phoxim, dimethoate, dichlorvos, chlorpyrifos and practical samples were used to validate the strip. Its sensitivity is in compliance with the OP residue testing standards in European Union (EU) and America. To the best of our knowledge, this is the first report of a hydrogel–agarose–Au NPs strip. Compared with the traditional methods, the proposed test strip is more economical and practicable with higher sensitivity and selectivity.

2. Experimental

2.1. Materials and instrumentation

Ten nanometers citrate-Au NPs (7.3 nM) was purchased from Shanghai Seebio Biotech. All the other chemicals were purchased from Alfa Aesar and Sigma–Aldrich at analytical grade and were used as received. A ZF-5-type UV-portable analyzer (Shanghai Jiapeng Technology Co., Ltd.) was used for the test strip fabrication. UV–vis spectra were recorded with a S3100 spectrophotometer (SCINCO). Transmission electron microscopy (TEM) images were obtained by using a H600 (HITACHI) model at an accelerating voltage of 75 kV. GC–MS analysis was performed on a Thermo Focus DSQ, equipped with a VF-5MS quartz capillary column (30 m, 0.25 mm inner diameter, 0.25 μm film thickness). The carrier gas was helium (99.999%), at a flow rate of 1 mL/min. Column temperature was initially 60 °C for 2 min, then gradually increased to 300 °C at 30 °C/min, kept there for 8 min. Electron ionization system was used with an ionization energy of 70 eV. 1.0 μL of the sample was injected automatically in splitless mode. Injector and detector temperatures were both set at 250 °C.

2.2. Preparation of reagent and sample solutions

A stock solution of acetylthiocholine (ATC) (1 mM) was freshly prepared in double-distilled water and used immediately to minimize possible hydrolysis. Phosphate-buffered saline (PBS) was used to prepare the AChE stock solution (1 unit/μL), which was diluted with distilled water and used immediately for the following experiments. 2% agarose solution was prepared with 2.5 mM $NaHCO_3$–Na_2CO_3 buffer (pH 9.0) and stored at 50–60 °C before used. Poly (ethylene glycol) diacrylate (PRGDA) was mixed with 2-hydroxy-2-methylpropiophenone (HMPP) at a volume ratio of 200:1 to generate the hydrogel precursor solution. A stock solution of RB (20 nM, 1.2 μL) was added into 10 nm citrate-Au NPs (7.3 nM, 0.5 mL), which were prepared with NaOH (2.5 mM) to get a pH of 9.5. The resulting solutions were kept in the dark at room temperature for 12 h. The fluorescence spectra of the RB–Au NPs solutions were measured with excitation at 550 nm [41]. All the OP compounds were initially dissolved in the methanol to prepared OP stock solutions (1 g/L), which was later diluted with double distilled water as needed. In the distilled solutions, such low levels of methanol have been shown negligible effects on the activity of AChE. River water that collected from Suzhou River in Shanghai urban area were used after static settlement and filtration. Different volumes of pesticide solutions were spiked into river water for test strip validation.

2.3. Fabrication of the test strip

The strip fabrication procedure was illustrated (Fig. 1A), well prepared RB–Au NPs (22.2 nM) and 2% agarose were both in water bath at 50–60 °C, then RB–Au NPs was mixed with agarose at a volume ratio of 4:1. The mixture was injected into a mold (a hollow cylinder with 7 mm inner diameter, 5 mm height) which was made of acrylic and stored at 4 °C for solidification. One end of absorbent paper (100 mm (length) × 10 mm (width) × 0.5 mm (thickness)) was saturated with 90% hydrogel precursor and exposed to UV irradiation (365 nm, 6 W) for 20 s to cure hydrogel and seal the paper. The end of strip was immerged into pH 9.0 buffer solution for 2 h. Then the solid agarose gel was attached on the end of strip by 15 μL 50% hydrogel precursor covered on gel and exposed to UV irradiation 10 s. The strip was stored in a sealed package before use.

2.4. Validation of RB–Au NPs functions in the test strip

ATC (40 μM) and AChE (15 mU/mL) were mixed together and incubated at 37 °C for 1 h to generate thiocholine solution. And, a series of diluent was prepared with pH 9.0 buffer solution. Then well prepared test strips were dipped into diluted thiocholine solutions (3 mL) and one was dipped into water as a blank control. All of the tests were maintained in the dark for about 2 h. Pictures were taken to show color changes. For UV–vis spectra measurement, gel attached on the strips were detached carefully, moved into a 0.5 mL Eppendorf (EP) tube which stored at 55 °C water to melt the gel. Then the melting gel was moved into a 250 μL cuvette and degassed in vacuum for 1 min to prepare for measurement of UV–vis absorption. For TEM imaging, gel were detached and crushed into paste. Red blank paste (made of blank gel) and color changed paste (made of color changed gel) were both added into pH 9.0 buffer solution (at ratio of 1:500), then prepared on copper grids and dried for TEM image.

2.5. Verification of the practical function of the test strip for OP detection

To find out a proper dosage of AChE used in OP pesticide detection, Chlorpyrifos, phoxim and dichlorvos were diluted at concentration of 0.10, 0.05 and 0.01 mg/L respectively. AChE was added in to diluent (final concentrations were set to be 10, 13, 16, 19, 18, 21, 25 mU/mL) and the resulting solutions were incubated at 37 °C for 1 h. 10 mU/mL AChE solution free of pesticides was used as the blank reference. Then each solution (5 mL) was finally added with ATC (final concentration at 40 μM) and a test strip. The reaction end was determined by the color change (from red to purple completely) of the blank. The pictures of the test strips were taken and UV–vis absorption of each strip gel was then measured by UV-Vis spectrometer. All the measurements were repeated six times.

To verify feasibility of the test strip, 16 mU/mL AChE was selected for all the experiments. Glyphosate, dimethoate, dichlorvos and chlorpyrifos were added into AChE solutions respectively and final concentrations of pesticides were set to be 0, 0.005, 0.01,