A highly sensitive, dual-signal assay based on rhodamine B covered silver nanoparticles for carbamate pesticides

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\section{1. Introduction}

Carbamate pesticides have been extensively used in modern agriculture because they have high insecticidal activity and short environmental persistence [1–4]. But they still have remain inevitably on vegetables, fruit and even in the water. Therefore, the residue of pesticides may have potential toxicity to human health [5–7]. Every year more than two million people died from pesticides poisoning [8]. Conventional analytical protocols used to determine carbamate pesticides are electrochemical analysis [9–12], gas chromatography, high performance liquid chromatography or couple to mass spectrometry [13–15]. Unfortunately, these analytical techniques suffered from the drawbacks of tedious pretreatment of samples, sophisticated instrumentation and require skilled operators [16]. Thus, these traditional methods are not suitable for on-site detection.

With the development of technology and nanoscience, nanomaterials have been thought to analysis pesticides on account of their minute size and unique chemical and physical properties [17]. Therefore, nanobiosensor techniques have made encouraging progress and attract increasing attention due to the advantages of easy operation, low cost, fast response and high sensitivity [18]. Most research works are based on the inhibition of carbamates pesticides on the activity of acetylcholinesterase (AChE) [19,20], mainly in terms of simplicity, rapidity, reliability, low detection limits, and cost-effectiveness. AChE and acetylcholine (ACh) are both important neurochemical substances in humans. AChE can hydrolyze ACh to generate thiocholine [21–23]. The presence of carbamate pesticides can inhibit the AChE activity and change the signal intensity. Thus it has become a simplified and portable alternative for pesticide detection.

In the literature [24], a highly sensitive and selective rhodamine B functionalized silver nanoparticles (RB-AgNPs) based assay with colorimetric and fluorometric for detecting carbamates pesticides have been reported. RB is an ideal ligand, and it covered on the surface of AgNPs via electrostatic interactions to cause the quenching of its fluorescence. The color changes are highly sensitive to the shape, size and medium refractive index. In our work, the aggregation of AgNPs has been used as colorimetric assay for pesticides. AChE and ACh added into RB-AgNPs solution lead to the color change of the mixture solution from yellow to gray, simultaneously accompanied by the recovery of fluorescence of RB.
The results showed that the enzyme inhibition rate was proportional to the carbaryl concentration in the range 0.001–1 μg/L with the detection limit of 0.32 ng/L. The fluorescence detection was more sensitive than UV–vis, which a linear calibration was obtained over the range of 0.1–8.0 ng/L with the limit of detection concentration was 0.023 ng/L. The proposed method showed a low detection limit, good selectivity and high reproducibility. By contrast, RB-AgNPs is much better sensitivity than those previously reported [25–28].

2. Results and discussion

RB-AgNPs were used to detect pesticides based on the aggregation of AgNPs which led to the color change. RB provides photostability, solubility and strong fluorescence. When RB adsorbs onto surfaces of AgNPs via electrostatic interaction, the fluorescence of RB is quenched. TEM illustrated the information on the shape and size of the prepared AgNPs. The TEM image indicated that the dispersed AgNPs (Fig. 1a) supported the color and spectral changes of the AgNPs. The sizes of the silver nanoparticles were about 20 nm. And the color of AgNPs solution on a pale yellow. Then, after the addition of ATCh and AChE, the TEM image indicated that amount of AgNPs had aggregated (Fig. 1b), and the color of the mixture became gray. After the addition of carbaryl, RB-AgNPs was back to dispersed state, and the color of RB-AgNPs remains yellow (Fig. 1c). These results could be also confirmed from the UV–vis absorption spectra. Both AChE and ATCh added into the RB-AgNPs solution, the fluorescence of RB molecules recovered, meanwhile, the color of mixture solution changed from yellow to gray. With the purpose of evaluating the potential of this dual-read assay for carbamate pesticides, carbaryl (10 μg/L) was added into AChE (1 unit/mL), put it at 37°C for 30 min. And then RB-AgNPs (0.4 mL, 10 nmol/L) and ATCh (10 μL, 20 μmol/L) were added into the mixture solution. We found that the color of the mixture remained yellow, and the fluorescence of RB remained quenched. It is because the fact that carbaryl can inhibit the activity of AChE and the generation of thiocholine was prevented. These phenomena was confirmed by UV–vis absorption and fluorescence spectra as shown in Fig. 2.

A linear regression model was constructed for carbaryl with the use of the UV–vis and fluorescence methods under the experimental conditions described above. The calibration parameters (Fig. 3b and c) indicate a good fit to the linear model. Fig. 3a shows the sensitivity for carbaryl by color change and fluorescence recovery. It is found that the color of the mixture solution still kept yellow after the addition of carbaryl in high concentrations. And when carbaryl in low concentrations added, the color of the mixture became gray. The UV–vis absorbance spectra and fluorescence spectra were used to demonstrate this situation. A linear calibration was obtained in the 0.001 μg/L–1 μg/L concentration range, and the correlation coefficient reached 99.3% with the limit of detection concentration for 0.32 ng/L [29]. The fluorescence assay is more sensitivity than UV–vis, which the concentration of carbaryl analyze is linear in range of 0.1–8 ng/L with the limit of detection concentration for 0.023 ng/L. In 2014, Li et al. [30] used unmodified silver nanoparticles to detect organophosphorus pesticide with a detection limit of 0.18 ng/mL. Kumar et al. [31] also proposed a similar method with the limits of detection of 2.402 nmol/L. In 2015. So the developed analysis is more sensitivity than those previously reported [32–34].
Lake water samples commonly contain many inorganic and organic substances, which may interfere with the analysis of the carbamate pesticides. Therefore, we did some interference experiments to investigate the interference effects of some inorganic ions and organic compounds that may coexist in the carbamate pesticides in samples, such as Cu²⁺, Fe³⁺, Al³⁺, Fe³⁺, Mn²⁺, Ca²⁺, Hg²⁺, K⁺, Na⁺, Mg²⁺, Al³⁺, PO₄³⁻, SO₄²⁻, Cl⁻, CO₃²⁻, vitamin C and vitamin B₂. The finally concentration of these ions were 20 μmol/L, which is 40 times to the standard concentration of carbaryl. As shown in Fig. 4, the resulting measurements showed that the effect of these ions were very minor.

Analytical application of RB-AgNPs for the detection of carbaryl in real samples was demonstrated through detecting carbaryl. In general, trace amounts of carbaryl in spiked in lake water, tomatoes and apples were detected. Lake water was collected from Runxi Lake in Nanchang University, Jiangxi. Tomatoes and apples were purchase from the supermarket in local city. The three samples were pretreated according to previous literatures [35]. The collected samples were filtered through to remove any particulate matters and then spiked with carbaryl (μg/L). Apples and tomatoes were stored at room temperature before use. Apple and tomato were chopped. Then, 5 g of samples were taken and then spiked with carbaryl (0, 0.01, 0.5 μg/kg) and stay for 30 min before extraction. To this, 10 mL of methanol was added and stirred for 10 min at room temperature. Then, Na₂SO₄ (0.2 mol/L, 1 mL) was added to sample solutions and kept for 2 min. The above solution was filtered and then analyzed by RB-AgNPs as a colorimetric sensor. The results obtained by standard addition method was summarized in Table 1. The recoveries of ultraviolet and visible spectrophotometer in the range from 85% to 130% were obtained. According to fluorescence spectrophotometer, the recoveries in the range was 90%–116%. The experimental results indicated that this sensor has the capability for detection of pesticides in real samples.

![Fig. 3](image-url) (a) Color change with decreasing concentrations of carbaryl from left to right (1–0.001 μg/L). (b and c) After addition of different concentration of carbaryl, a linear regression model was constructed. The UV-vis and fluorescence methods were under the optimization condition.

![Fig. 4](image-url) The selectivity of RB-AgNPs, 0 is control, 1–17 is all kinds of inorganic and organic compounds (Cu²⁺, Fe²⁺, Al³⁺, Fe³⁺, Mn²⁺, Ca²⁺, Hg²⁺, K⁺, Na⁺, Mg²⁺, Al³⁺, PO₄³⁻, SO₄²⁻, Cl⁻, CO₃²⁻, vitamin C and vitamin B₂).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked</th>
<th>UV-vis</th>
<th>Fluorescence</th>
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<tr>
<td></td>
<td></td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>Recovery</td>
<td>RSD</td>
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<tr>
<td>Lake water¹</td>
<td>0</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
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<td>0.013</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.62</td>
<td>124</td>
</tr>
<tr>
<td>Tomato²</td>
<td>0</td>
<td>/</td>
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<td>0.009</td>
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¹ Spiked (μg/L), Found (μg/L), Recovery (%), relative standard deviation; RSD (%).
² Spiked (μg/kg), Found (μg/kg), Recovery (%), relative standard deviation; RSD (%).
3. Conclusion

In summary, we used the RB-AgNPs (which have colorimetric and fluorometric) to detect carbamate pesticides in real samples. And it is based on the color change of RB-AgNPs. If the color of solution remains yellow, it indicates that it has pesticides residual. When the color of solution becomes gray, it has no pesticide. This sensor provides convenience for testing real samples. The proposed method was successfully applied to in real samples by distinguished the color change by our naked eye. We believed that this method can be useful in detecting pesticides residual.

4. Experimental

4.1. Reagents and chemicals

Silver nitrate (AgNO₃), sodium citrate dehydrate (Na₃C₆H₅O₇·2H₂O), sodium borohydride (NaBH₄, 96%) and rhodamine B were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Acetylcholinesterase (ACHE, 744 unit/mg) and acetylthiocholine iodide (ATCh) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris–HCl buffer (pH 7.5) was used to dissolve ACHE (1 unit/mL). The solution of ATCh (10 mmol/L) was freshly prepared in double distilled water and it cannot be used for more than 3 h. Carbaryl was purchased from Chem Service, West Chester, PA, USA. Carbaryl was used without further purification and dissolved in methanol. All of solutions used in the experiments were stored at 4°C. And used immediately for the following experiments. All chemical used were Analytical Grade reagents, and all glassware was cleaned by aqua regia and double-distilled water more than three times. Water samples were collected from the local pools in Nanchang. And tomato and apple were purchased from supermarket in Nanchang.

4.2. Instrumentation

UV–vis absorption spectra were recorded on an Agilent 8453 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). FL emission spectra were obtained by an LS-55 luminescence spectrometer (Perkin Elmer Co., MA, USA). Transmission electron microscopy (TEM) measurements were made on JEM-2100 (JEOL Co., Japan). The associated point and linear resolutions were 0.23 nm and 0.14 nm, respectively, and the operational accelerating voltage was 200 kV. The samples for TEM characterization were prepared by placing a drop of solution on a carbon coated copper grid and dried at room temperature.

4.3. Preparation of AgNPs and RB-AgNPs

AgNPs were prepared according to the literature [30]. AgNO₃ (250 μL, 100 mmol/L) solution was added into 100 mL double distilled water in a 250 mL flat bottomed flask immersed in an ice-water bath (5°C) with vigorous stirring, then, sodium citrate solution (250 μL, 100 mmol/L) and sodium borohydride (6 mL, 5 mmol/L) solution were sequentially added to the solution. The reaction was quite fast, and the colorless reactant mixture immediately turned pale yellow; this indicated the formation of dispersed, colloidal AgNPs. The mixture was rapidly stirred for a further 30 min, and the resulting product was then stored in the dark for 24 h before further use. The size and shape of AgNPs were characterized by TEM, and its absorption spectrum was recorded with the use of the UV–vis spectrophotometer. RB-AgNPs were prepared as below: RB solution (0.6 mL, 2 mmol/L) was added into solutions of the AgNPs (1 mL, 5 mmol/L) with stirring. Then the resulting solutions were stirred slowly in the dark at room temperature for 2 h. The fluorescence spectra of the RB-AuNPs solutions were measured with excitation at 580 nm. According to fluorescence spectra, the very weak fluorescence of RB-AuNPs solutions indicated that almost all the RB molecules had absorbed onto the surfaces of AuNPs. This suggests that the fluorescence was strongly quenched by AgNPs.

4.4. Detection of carbaryl with RB-AuNPs using AChe and ATCh

A suitable amount of the carbaryl standard solutions were added into the AChe solution (1 unit/mL). After stay these mixture solution at 37°C for 30 min, RB-AgNPs (0.4 mL, 10 mmol/L) was added into each mixture solution. Finally, ATCh (10 μL, 20 μmol/L) were added into the mixtures. After the resulting solution was kept in the dark room for 5 min, UV–vis absorption and fluorescence were measured respectively.

Acknowledgment

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