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Highly sensitive colorimetric detection of organophosphate pesticides using copper catalyzed click chemistry

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ABSTRACT

Highly sensitive colorimetric detection of organophosphate pesticides (OPs) was developed using Cu (I)-catalyzed click chemistry as the colorimetric signal amplification process between the acetylcholine esterase–acetylthiocholine system (AChE–ATCl) and azide- terminal alkyne-functionalized Au NPs as the colorimetric probe. It was demonstrated that the involvement of Cu (I)-catalyzed click chemistry allowed greatly improved colorimetric sensitivity for OPs detection based on the indirect modulation of click chemistry-induced Au NPs aggregation by the AChE–ATCl system. Paraoxon as the model OPs in the concentration range from 10^{-6} to 10^{-4} g/L can be directly detected using the naked-eye-based colorimetric assay without the aid of any complex instruments. The results for paraoxon detection in spiked apple juice were found to be in good agreement with that obtained by the conventional UV–vis spectroscopy. This simple and reliable assay would greatly improve the public safety and environmental protection in an on-site and real-time detection format.

1. Introduction

Organophosphate pesticides (OPs) have been the most popular insecticides applied in agriculture all over the world. Due to the widespread use of OPs, their residues and metabolites in food, water and soil currently represents one of the major issues for the environmental chemistry [1–3]. It has been well established that the irreversible inhibition of acetylcholine esterase (AChE) by OPs even at low concentrations can lead to serious clinical complications and ultimately result in death. An effective strategy for dealing with OPs contamination in the environment has to commence with an assessment of the extent of the problem.

Conventional analytical methods, such as chromatography–mass spectrometry and enzyme-linked immunosorbent assay tests [4–6], are very powerful tools for the detection of OPs. However, these methods are not adapted for in-situ and real-time detection attributed to their dependence on complex instruments, well-equipped laboratories, highly trained personnel and costly analytical procedures. Attacking this problem head on, generally applicable assays based on simple readout methods are much sought after for public safety and environmental protection [7,8]. Recently, the naked-eye-based colorimetric readout methods have showed great potential for analytical detection in terms of their miniaturization, throughput and no aid of complex instruments [9,10].

To meet the demand of naked-eye-based readout methods, various colorimetric probes have been developed. As a promising colorimetric probe supporting naked-eye-based readout methods, gold nanoparticles (Au NPs) even at nanomolar concentrations show observable red-to-blue color change upon nanoparticle aggregation due to their high extinction coefficient [11–13]. Detection of OPs based on the direct stimulation of Au NPs aggregation or growth by the acetylcholine esterase–acetylthiocholine system (AChE–ATCl) has been reported [14,15]. However, high amounts of OPs are usually required to initiate detectable color change of Au NPs because of the direct dependence of nanoparticle aggregation on the AChE–ATCl system [16], limiting the colorimetric sensitivity. Therefore, an intermediate colorimetric signal amplification process between the AChE–ATCl system and the probe is highly required.

Click chemistry has emerged as a high-yield and highly selective chemical approach attracting great attention in many fields. Cu (I)-catalyzed click chemistry between azides and alkynes [17,18] are particularly attractive in the field of sensor due to its highly sensitive and selective nature [10]. The fact that only a trace amount of Cu^{+} is required as the catalyst for the efficient conjugation between azide and alkyne groups provides an important copper-mediated signal amplification effect [19]. Accordingly, based on the indirect monitoring of copper ions, even a minor change in the amount of copper ions could dramatically influence the click chemistry-mediated readout, leading to greatly amplified detection sensitivity [10].

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Herein, the highly sensitive colorimetric detection of OPs was developed using Cu (I)-catalyzed click chemistry with azide- and terminal alkyne-functionalized Au NPs as the colorimetric probe. As is shown in Scheme 1, Cu^{+} required as the catalyst for cycloaddition between azide and terminal alkyne groups on surfaces of Au NPs is released from CuO NPs by CH_3COOH enzymatically produced from the AChE–ATCl system in the presence of reductant (sodium ascorbate). OPs leads to the inhibition of AChE activity, which subsequently blocks the production of CH_3COOH needed for the release of copper ions. Significantly, due to the copper-mediated signal amplification effect, even a negligible change in the amount of Cu^{+} could greatly influence the click chemistry efficiency between azide and terminal alkyne groups on surfaces of Au NPs. As a result, a greatly amplified red-to-blue colorimetric signal induced by the aggregation of Au NPs is associated with the concentration of OPs. Hence, Cu (I)-catalyzed click chemistry acted as the intermediate colorimetric signal amplification process between the AChE–ATCl system and the probe. More importantly, the detection procedure can be carried out in an on-site and real-time format by the naked-eye-based readout method without the aid of any complex instruments.

2. Experiment

2.1. Reagent and materials

Azide- and terminal alkyne-functionalized Au NPs solutions were prepared according to the previous work [10,19]. Acetylcholine esterase (AChE, Electriceel 686 U/mg), acetylthiocholine (ATCl) and sodium ascorbate were purchased from Sigma (USA). CuO NPs (40 nm) were obtained from Adamas Reagent Co., Ltd (Switzerland). Apples used for the detection of OPs in real samples were purchased from local supermarket. Unless otherwise stated, reagents were of analytical reagent grade and used as received. Aqueous solutions were prepared with deionized water (18.2 MΩ cm) from a Milli-Q purification system.

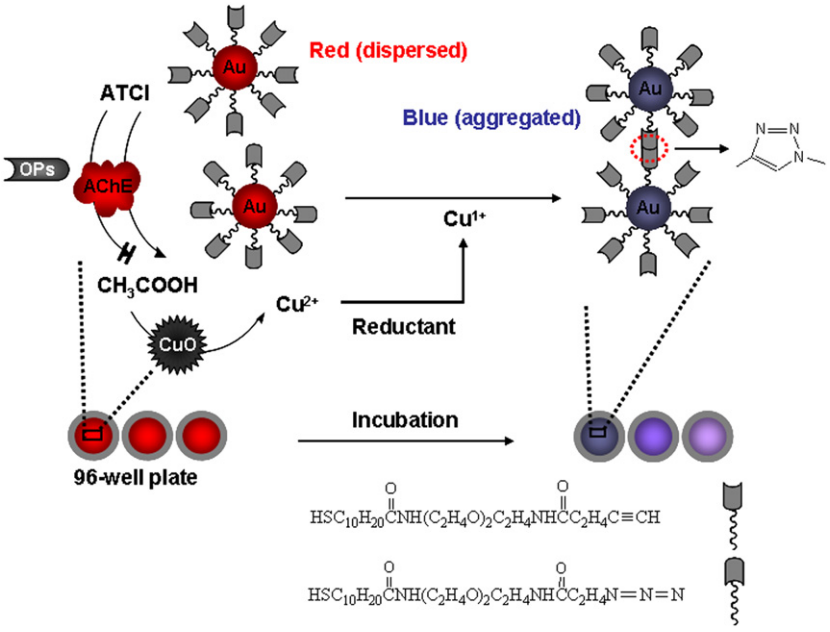
2.2. Apparatus

UV–vis absorption spectra were recorded on an Infinite M200 NanoQuant microplate reader (Tecan Group Ltd., Switzerland). Transmission electron microscopy (TEM) measurements were made on a Tecnai G^2 20 TWIN transmission electron microscope (FEI Co., USA). Photographs were taken by a Panasonic GF2WGK digital camera (Japan).

2.3. Detection protocol

AChE solutions were prepared with 0.01 M PBS (pH 7.4) and were stored at 4 °C when not in use. CuO NPs were dispersed in deionized water with ultrasonication for 30 min, and were used immediately after the sonication. Standard paraoxon solutions were prepared by dispersing the stock acetone solution of paraoxon in 0.01 M PBS (pH 7.4) with ultrasonication for 10 min. The azide- and terminal alkyne-functionalized Au NPs solutions were equally mixed to obtain the probe solutions.

The detection was carried out in a 96-well plate. 180 μL of the probe solution was first added in a well of a 96-well plate,



Scheme 1. Naked-eye-based colorimetric detection of OPs using copper catalyzed click chemistry (OPs: organophosphate pesticides; Au: gold nanoparticles; AChE: Acetylcholine esterase; ATCl: acetylthiocholine; CuO: CuO nanoparticles).

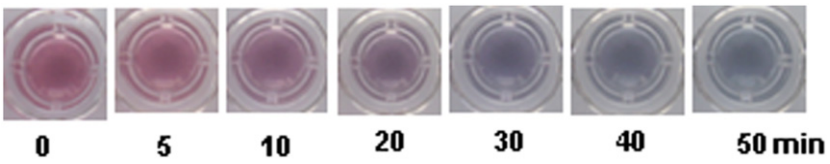


Fig. 1. Photographs of the uninhibited detection system during the incubation process within 50 min.

followed by the successive addition of 5 μL CuO NPs suspension, 5 μL sodium ascorbate aqueous solution (0.02 M) and 5 μL ATCl aqueous solution. Finally, 5 μL AChE solution (0.05 mg/mL), pre-inhibited by incubating the equal mixture of 0.1 mg/mL AChE solution and PBS with or without paraoxon for 30 min at room temperature, was added in the well. After thorough mixing, the 96-well plate was placed on a breaker for incubation. The color of the detection system in the well was recorded with a digital camera at intervals during incubation. UV–vis spectroscopic and transmission electron microscopic measurements of the detection system were also made before and after the incubation process.

2.4. Detection of paraoxon in spiked apple juice

5.0 g edible parts of an apple were chopped and crushed into a homogenate with the addition of 20 mL 0.01 M PBS (pH 7.4). After stirring of the homogenate for 60 min, the homogenate was centrifuged for 15 min at 3000 rpm. The precipitate was discarded to collect the apple juice. Different concentrations of standard paraoxon solutions were then added in the apple juice to obtain the spiked apple juice with the final paraoxon concentrations of 5×10^{-6} and 5×10^{-5} g/L. The concentration of paraoxon in the spiked apple juice was determined both by the naked-eye-based colorimetric method and UV–vis spectroscopy according to the detection protocol except the replacement of the standard paraoxon with the spiked apple juice.

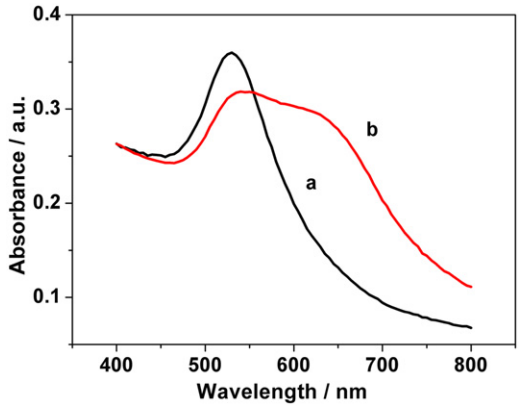


Fig. 2. UV–vis spectroscopy of the uninhibited detection system before (a) and after (b) the incubation process for 40 min.

3. Results and discussion

3.1. Characterization of the colorimetric detection system

The color change of the detection system during incubation was first studied using the uninhibited AChE–ATCl system. Factors that could influence the color change of the detection system such as concentrations of CuO NPs, ATCl and AChE were optimized (For details of optimization of the conditions, see Figs. S1 and S2 in the supplementary materials). Fig. 1 shows the color change of the detection system during incubation for 50 min. Excitingly, under optimized experimental conditions, the detection system showed an obvious gradual change in color from red to blue within 40 min during the incubation process. The color change can be clearly observed by the naked eye alone.

UV–vis spectroscopy and TEM were then used to investigate the mechanism of the color change of the detection system. Fig. 2 shows the UV–vis spectroscopy of the detection system before (Fig. 2a) and after (Fig. 2b) the incubation process. It was found that the plasmon band of Au NPs at 530 nm red-shifted to 625 nm after the incubation process. This phenomenon was similar to that induced by the aggregation of Au NPs [10]. It has been well established that the aggregation of Au NPs even at low concentrations can display a clear red-to-blue color change.

As expected, TEM verified the aggregation of Au NPs during the incubation process as shown in Fig. 3. The pure mixture of azide- and terminal alkyne-functionalized Au NPs with stable red color dispersed well during incubation (Fig. 3A), while the detection system with red-to-blue color change exhibited obvious Au NPs aggregation after the incubation process (Fig. 3B). These results indicated that the color change of the detection system can be attributed to the aggregation of Au NPs during the incubation process.

3.2. Mechanism of the colorimetric detection of OPs

To study the mechanism of aggregation of Au NPs during the incubation process, the performance of components in the detection system was investigated. Wells of the 96-well plate with the addition of different components in the detection systems were monitored during the incubation process. Fig. 4 shows the photographs of detection systems with different components after the incubation process. Well A1 with only the probe showed stable red color during incubation. No color change was observed after the addition of CuO NPs to the probe (A2), excluding the direct inducement of Au NPs aggregation by CuO NPs. In the presence of CuO NPs, the color remained unchanged upon further

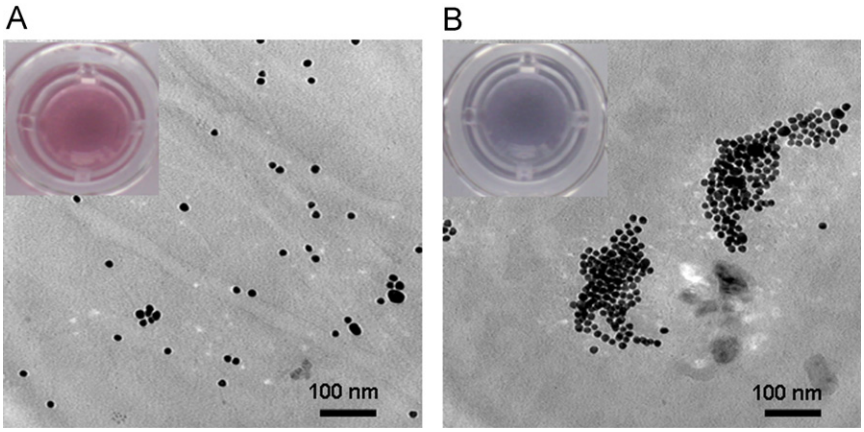


Fig. 3. TEM of the pure mixture of azide- and terminal alkyne-functionalized Au NPs (A) and the uninhibited detection system after the incubation process for 40 min (B). Inset: photographs of the corresponding detection systems.

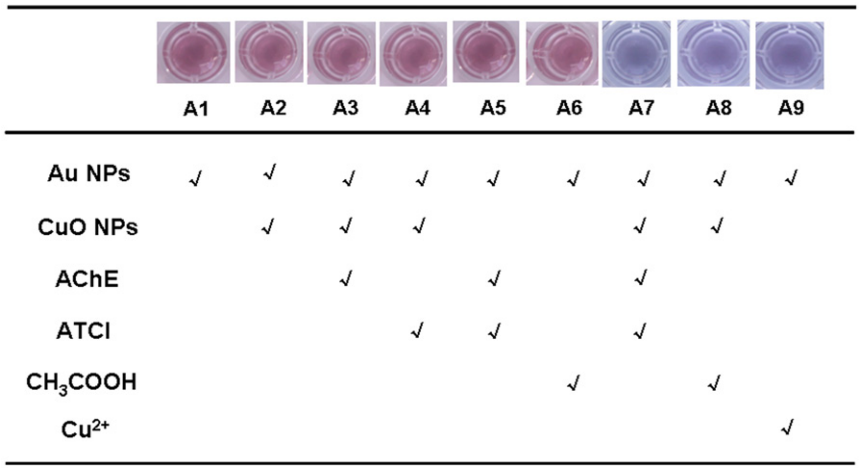


Fig. 4. Photographs of the uninhibited detection systems with different components after incubation for 40 min. Components added in the detection systems were indicated below the photographs. Sodium ascorbate solution was added in each well.

addition of AChE or ATCl (A3, A4), indicating the requirement of the integral AChE–ATCl system which could enzymatically produce CH_3COOH. On the contrary, well A5 containing the integral AChE–ATCl system still showed no color change in the absence of CuO NPs, in agreement with well A6 with the direct addition of CH_3COOH in the absence of CuO NPs. The result implied the need of CuO NPs as the resource of the catalyst, and excluded the direct inducement of Au NPs aggregation by the AChE–ATCl system and CH_3COOH. Significantly, positive result was observed only upon the addition of both the integral AChE–ATCl system and CuO NPs (well A7).

It was reasoned that CH_3COOH enzymatically generated from the AChE–ATCl system dissolved CuO NPs to release Cu^+ in the presence of reductant. Cu^+ then typically catalyzed the conjugation between azide and terminal alkyne groups on surfaces of Au NPs, leading to the aggregation of Au NPs. Positive results of well A8 with CuO NPs and CH_3COOH, and well A9 with Cu^{2+} further supported the above mechanism. As a result, the AChE–ATCl system indirectly modulated the aggregation of Au NPs through the release of copper ions as the catalyst for click chemistry between azide and terminal alkyne groups on surfaces of Au NPs. Because of the dependence of AChE activity on OPs, the concentration of OPs might be visualized by the aggregation-induced red-to-blue color change of Au NPs based on the indirect modulation of release of copper ions needed as the catalyst for click chemistry.

3.3. Colorimetric detection of paraoxon

To study the performance of the assay in colorimetric detection of OPs, the OPs-inhibited detection systems were monitored with this naked-eye-based colorimetric method using paraoxon as the typical model OPs. AChE was pre-inhibited by paraoxon of different concentrations for 30 min before addition in the detection systems for incubation.

Remarkably, these detection systems exhibited clearly different rate of color change from red to blue during the incubation process. Fig. 5 shows the photographs of the detection systems with AChE pre-inhibited by different concentrations of paraoxon during incubation for 80 min. As can be seen, the higher the concentration of paraoxon, the slower the red-to-blue color change of the detection system. It has been well established that the enzymatic activity of AChE can be irreversibly inhibited by OPs even at low concentrations. Higher OPs concentration could lead to lower AChE activity. Therefore, the concentration-dependant inhibition of AChE activity by paraoxon suppressed the production of CH_3COOH needed for the release of copper ions, and subsequently blocked the aggregation-induced red-to-blue color change of Au NPs attributed to the Cu (I)-catalyzed conjugation between azide and terminal alkyne groups on surfaces of Au NPs. Color difference of these detection systems was highly observable to the naked eye after incubation for 30 min, which regularly changed with the increase of incubation time. Using this process of color change, paraoxon in the concentration range from 10^{-6} to 10^{-4} g/L could be directly detected with the naked eye without the aid of any complex instruments, which was enough to satisfy current detection requirement according to the

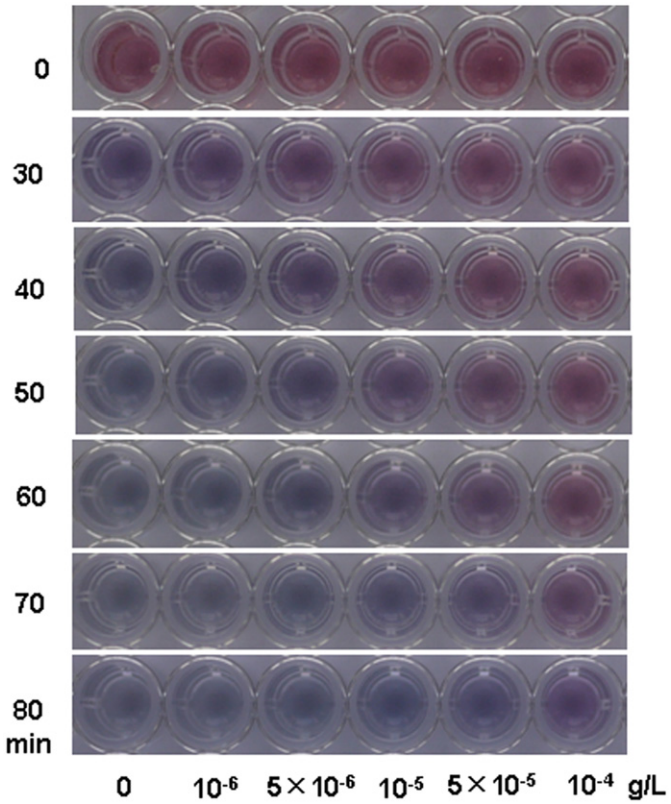


Fig. 5. Photographs of the detection systems with AChE pre-inhibited by 0, 10^{-6}, 5×10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4} g/L paraoxon during incubation within 80 min.

pesticide residue standard of the European Union (paraoxon, 2×10^{-5} g/L). More importantly, the lowest detectable paraoxon concentration of this assay (10^{-6} g/L) by the naked eye was about one order of magnitude lower than the previously reported value (1.2×10^{-5} g/L) [14]. Due to the copper-mediated signal amplification effect, even a minor change in release of copper ions induced by OPs could greatly influence the click chemistry efficiency between azide and terminal alkyne groups on surfaces of Au NPs, resulting in greatly amplified color change of the probe. Hence, Cu (I)-catalyzed click chemistry acted as the intermediate signal amplification process to improve the colorimetric sensitivity.

In addition, conventional UV–vis spectroscopy was used to confirm the feasibility of the naked-eye-based colorimetric assay. Fig. 6 shows the UV–vis spectroscopy of these detection systems after incubation for 40 min. It was found that the increase of paraoxon concentration resulted in obvious increase in the absorbance at 530 nm (A530) and concomitant decrease in the absorbance at 625 nm (A625). It has been well known that the aggregation of Au NPs could lead to obvious significant red-shift of the plasmon band of Au NPs. The ratio between A625 and A530 was proportional to the logarithm of paraoxon concentration ($r=0.9799$) in the range from 10^{-6} to 10^{-4} g/L. The detection limit of paraoxon using UV–vis spectroscopy was 10^{-6} g/L. The result further demonstrated the feasibility of the naked-eye-based colorimetric assay for paraoxon detection.

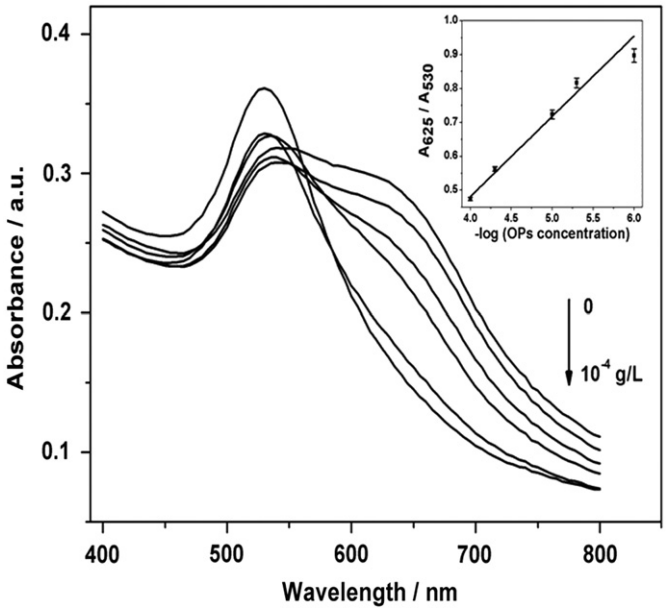


Fig. 6. UV–vis spectroscopy of the detection systems with AChE pre-inhibited by 0, 10^{-6}, 5×10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4} g/L paraoxon after incubation for 40 min. Inset: calibration plot of A625/A530 vs. −log (paraoxon concentration).

3.4. Detection of paraoxon in spiked apple juice

To evaluate the utility of the naked-eye-based colorimetric assay for OPs detection in real samples especially agricultural products containing many interferents, paraoxon residue in spiked apple juice was determined with both this naked-eye-based colorimetric method and conventional UV–vis spectroscopy. Apple juice samples extracted with PBS were added with standard paraoxon solutions. AChE pre-inhibited by the spiked apple juice was used in the detection systems. Fig. 7 shows the detection results obtained with both this colorimetric method and conventional UV–vis spectroscopy. Detection results of the UV–vis spectroscopy were obtained according to the linear relationship of the standard paraoxon in Fig. 6. Results obtained by both this colorimetric method and UV–vis spectroscopy corresponded well with that of standard paraoxon in Figs. 5 and 6. Furthermore, the determined concentrations of paraoxon in the spiked apple juice by UV–vis spectroscopy were in good agreement with the added standard concentrations with acceptable recovery. These results indicated an acceptable feasibility of the naked-eye-based colorimetric assay for OPs detection in real complex samples especially agricultural products containing many interferents.

4. Conclusions

A highly sensitive naked-eye-based colorimetric assay for OPs detection was developed using Cu (I)-catalyzed click chemistry with azide- and terminal alkyne-functionalized Au NPs as the colorimetric probe. Based on the indirect modulation of Au NPs aggregation by the AChE–ATCl system through click chemistry between azide and terminal alkyne groups on Au NPs surfaces, Cu (I)-catalyzed click chemistry acted as the intermediate colorimetric signal amplification process between the AChE–ATCl system and Au NPs. The involvement of click chemistry greatly improved the colorimetric sensitivity for OPs detection. More significantly, the assay can be carried out in an on-site and real-time format by the naked-eye-based readout method without the aid of any complex instruments. This simple and reliable assay would greatly improve the public safety and environmental protection.

Added standard paraoxon (g/L)	Determined photograph and concentration (g/L) by the Naked eye	Determined paraoxon by UV-vis spectroscopy (g/L)	Recovery (%)
5×10^{-5}	 5×10^{-5}	$(5.25\pm0.40)\times10^{-5}$	104.9±8.1
5×10^{-6}	 5×10^{-6}	$(4.60\pm0.46)\times10^{-6}$	92.30±9.1

Fig. 7. Detection results of paraoxon in spiked apple juice with both the naked-eye-based colorimetric method and conventional UV–vis spectroscopy. Photographs and UV–vis spectroscopy were recorded after the incubation process for 50 min and 40 min, respectively.

Acknowledgments

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.10.016.

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