Analyst

PAPER

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Cite this: *Analyst*, 2016, **141**, 1837

Pesticide analysis using nanoceria-coated paper-based devices as a detection platform†

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Received 20th November 2015,
Accepted 18th January 2016

DOI: 10.1039/c5an02403j

www.rsc.org/analyst

We report the first use of a paper-based device coated with nanoceria as a simple, low-cost and rapid detection platform for the analysis of organophosphate (OP) pesticides using an enzyme inhibition assay with acetylcholinesterase (AChE) and choline oxidase (ChOX). In the presence of acetylcholine, AChE and ChOX catalyze the formation of H_2O_2, which is detected colorimetrically by a nanoceria-coated device resulting in the formation of a yellow color. After incubation with OP pesticides, the AChE activity was inhibited, producing less H_2O_2, and a reduction in the yellow intensity. The assay is able to analyze OP pesticides without the use of sophisticated instruments and gives detection limits of 18 ng mL^{-1} and 5.3 ng mL^{-1} for methyl-paraoxon and chlorpyrifos-oxon, respectively. The developed method was successfully applied to detect methyl-paraoxon in spiked vegetables (cabbage) and a dried seafood product (dried green mussel), obtaining ~95% recovery values for both sample types. The spiked samples were also analyzed using LC-MS/MS as a comparison to the developed method and similar values were obtained, indicating that the developed method gives accurate results and is suitable for OP analysis in real samples.

Introduction

Pesticides are well known human toxins used in agriculture to protect plants from insects and pests during the production and post-harvest storage of crops to increase agricultural yields. As a result of the widespread and long-term use of pesticides, contamination of both environmental[1,2] and food samples[3,4] has occurred. Of many types of pesticides, organophosphate (OP) pesticides are among the most common as they have relatively low persistence under natural conditions and are highly effective for pest and insect control. However, these toxic compounds can affect human health when the parent compound or its residues are present in food[5] or water.[1] The toxicity of OP is associated with their capacity to irreversibly inhibit acetylcholinesterase (AChE) activity in the central and peripheral nervous systems, causing an accumulation of the neurotransmitter acetylcholine in the body, resulting in organ and nervous system failure and potentially death.[6–9] Therefore, sensitive and selective detection methods for OP are highly desirable due to the concerns of public safety and environment protection.

Several methods have been developed for quantitative analysis of OP pesticides, including high-performance liquid chromatography (HPLC),[10–12] liquid/gas chromatography-mass spectrometry (LC-MS/GC-MS),[13,14] electrochemical analysis,[15–19] and enzyme-linked immunosorbent assays (ELISAs).[20–22] Although these methods provide effective analysis with high sensitivity and detection limits at the nanomolar level, they are time-consuming, costly, rely on complicated instruments, require highly trained personnel, and/or are difficult to use on-site. Recently, enzyme inhibition assays have been developed for a simpler detection of OP.[23–26] The assay is based on the inhibition of AChE activity by OP pesticides. The decrease in AChE activity can be measured by colorimetric,[27] fluorescence,[28] electrochemistry[29–31] and/or chemiluminescence assays[29] to determine pesticide concentration. However, these aforementioned methods still require expensive instruments, long analysis times, and/or large amounts of reagents and samples[32] making them less portable than desired for on-site analysis.[27,33,34] The combination of enzyme inhibition assays and nanoparticle-based colorimetric assays have also been introduced for a rapid detection of OP.[34] However, this method suffered from an uncontrolled aggregation of nanoparticles in real samples, leading to poor selectivity.

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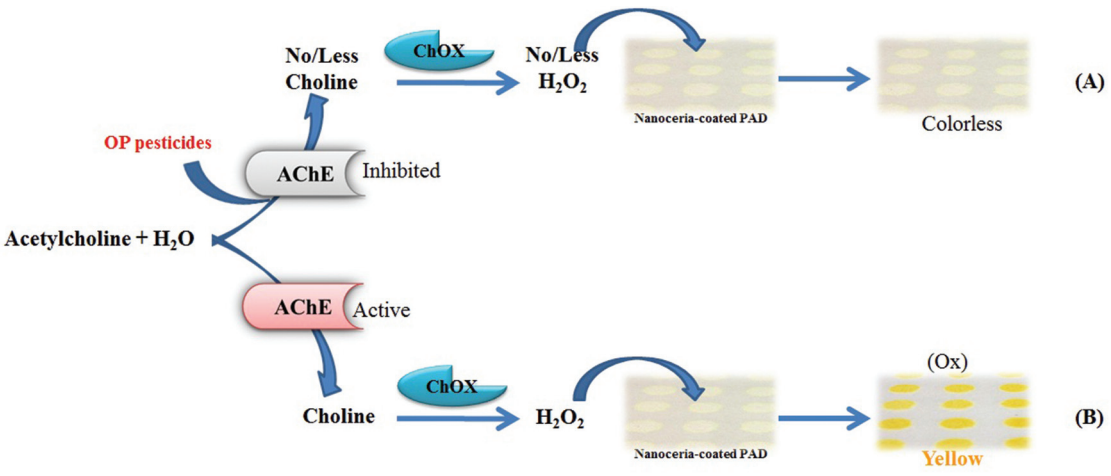
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5an02403j

Paper-based analytical devices (PADs)[35,36] are known as an alternative method for point-of-care diagnostics and chemical analysis in fields such as urinalysis,[37] food safety,[38] environmental monitoring,[39] and biochemical analysis.[40] PADs are attractive because they are simple, low-cost, lightweight, portable, easy to fabricate and use, disposable and provide timely results.[35,36,41] Recently, PADs in a dipstick format have been applied for pesticide analysis using an AChE inhibition assay where the product of AChE-catalyzed hydrolysis was quantified using chromogenic substrates including indophenyl acetate (IPA),[4] dithiobisnitrobenzoate (DTNB),[42] and indoxyl acetate.[43] Color development was visualized by both the naked eye for a yes/no answer and quantitatively analyzed using a handheld reader or a cell phone.[41,43] However, these colorimetric assays still required a large amount of chromogenic reagent and enzyme (>0.5–5 unit of AChE per assay).[4,42] Furthermore, the assays required multiple analysis steps and quantification relied on the color and intensity interpretations that could be influenced by ambient lighting and device dryness.[27,44] In addition, chromogenic reagents used in previous work (DTNB) are unstable and light sensitive.[45] Finally, these paper dipsticks have not been applied for pesticide analysis in complex matrix samples such as foods and vegetables.

Nanoceria (ceria nanoparticles, CeO_2 NPs) has been used in a variety of applications such as biomedicine, environmental monitoring, solid oxide fuel cells, microelectronics, catalysis and sensing[46] because it is auto-catalytic, has free radical scavenging properties and low toxicity.[47] Nanoceria also has oxidase-like properties in aqueous environments as they can catalyze the oxidation reaction of H_2O_2 with peroxidase substrates.[48] Recently, a direct detection of H_2O_2 using nanoceria as colorimetric agents on a paper-based device was reported.[49] The analysis is based on changing the Ce^{3+}/Ce^{4+} ratio on the nanoceria surface by H_2O_2 resulting in the color change from colorless to yellow.[47,50] The amount of H_2O_2 present in the samples can be directly quantified by nanoceria without the need of peroxidase enzyme and organic dye substrates. However, only a few applications have been demonstrated for the use of nanoceria for chemical analysis.[45]

Here, a paper-based device for the determination of OP pesticides using enzyme inhibition and nanoceria is reported. It is the first time that the paper-based device has been applied for the determination of OP pesticides in complex food sample matrices. The general procedure for the assay is described in Scheme 1. AChE and ChOX catalyze the formation of H_2O_2 in the presence of acetylcholine. The H_2O_2 reacts with nanoceria predeposited on the paper resulting in the formation of a yellow color that can be observed by the naked eye (Scheme 1B) and/or quantified using an office scanner and image processing software. In the presence of OP pesticides, the activity of AChE is inhibited leading to a decrease in the yellow intensity (Scheme 1A). Here, we first evaluated the use of nanoceria-coated paper-based device for analysis of H_2O_2. The results showed that high sensitivity with low detection limits could be achieved under optimized conditions. The enzymatic assay was then optimized for AChE, acetylcholine, ChOX and organic solvent concentration using two standard OP pesticides, methyl-paraoxon (MPO) and chlorpyrifos-oxon (CPO). The sensitivity, linear range and detection limit of the assay against two OP standards were found to depend primarily on the AChE concentration. Finally, to test the assay performance in complex sample matrices, OP spiked cabbage and dried green mussel samples were analyzed. Similar results were obtained using the PAD assay and LC-MS/MS indicating that the enzymatic assay with nanoceria-coated paper can be applied for a selective determination of OP pesticide in complex samples. We believe that the paper-based device in this format provides a cost-effective approach for OP analysis. For the developed 5 mm diameter single spot test, the total



Scheme 1 Paper-based device for the analysis of OP pesticides using enzymatic assay and nanoceria as colorimetric agents. (A) Upon the addition of OP pesticides, AChE activity is inhibited resulting in no/less H_2O_2 and hence less nanoceria color developed. (B) Without the addition of OP pesticides, AChE activity is normally active producing H_2O_2 that causes the color change of nanoceria.

cost estimate is approximately $0.0341 including the device fabrication cost and reagent cost (Tables S1 and S2†).

Experimental

Chemicals and materials

All chemicals were analytical grade. Acetylcholinesterase (AChE) (E.C.3.1.1.7), choline oxidase (ChOX) (E.C.1.1.3.17), acetylcholine chloride (ACh), bovine serum albumin (BSA), nanoceria solution or cerium(IV) oxide nanoparticles (CeO_2 NPs, 20% w/v), polyethylene glycol (PEG, 6000 g mol^{-1}), hydrogen peroxide (H_2O_2), methanol, methyl-paraoxon and chlorpyrifos-oxon were purchased from Sigma-Aldrich. Tris (hydroxymethyl) aminomethane was ordered from Omnipur, China. Toluene was purchased from RCI Labscan, Thailand. Dispersive solid phase extraction (SPE) devices with a total volume of 2 mL (5982–4921) were ordered from Agilent Technologies. Polystyrene was purchased from a local stationary shop, Chon Buri, Thailand. Whatman no. 4 filter paper was purchased from Whatman International Ltd, China. The patterned screen was obtained from a local screen-printing shop, Chon Buri, Thailand. Deionized water (DI) prepared by the Barnstead™ e-pure™ ultrapure water purification system was used throughout the experiments.

The solutions including nanoceria, 10 mM of Tris buffer pH 7.4 and 10 mg mL^{-1} of PEG were prepared in DI water and stored at 2–8 °C until use. Stock solutions of 50 mg mL^{-1} of methyl-paraoxon and 10 mg mL^{-1} of chlorpyrifos-oxon were dissolved in methanol and stored at 2–8 °C until use. Working pesticide solutions were prepared daily by a dilution of the stock solution using 4% (v/v) methanol in water. BSA (10 mg mL^{-1}) and acetylcholine (100 mM) stock solutions were prepared in Tris buffer (pH 7.4) and stored at 2–8 °C until use. Stock solutions of 1000 U mL^{-1} of AChE and 100 U mL^{-1} of ChOX were prepared in the BSA solution and stored at −20 °C until use. Working solutions of acetylcholine, AChE and ChOX were daily prepared by a dilution of the stock solution using BSA solution.

Fabrication of nanoceria-coated paper-based device

Paper-based devices were fabricated using a previously reported polymer screen-printing method.[51] Briefly, polystyrene solution (25% w/v in toluene) was applied onto a patterned screen placed on top of a Whatman #4 filter paper as the hydrophobic barrier. The solution passed through the patterned screen and penetrated through the paper to create a hydrophobic barrier with a circular test zone of a 5 mm diameter (Fig. S1†). A clear packing tape was adhered on the back side of the device to prevent leaking.[52] The patterned paper devices were then coated with nanoceria by depositing 5 μL of 3% w/v colloidal nanoceria solution onto test zones and allowed to dry. Unfortunately, nanoceria particles were hydrophobic after drying, preventing wetting in the detection zone as shown in our previous work.[51] To increase hydrophilicity of the detection zone, 5 μL of 10 mg mL^{-1} PEG solution was added to the device.[53] After drying, the nanoceria-coated PADs were ready for colorimetric detection of OP pesticides.

H_2O_2 analysis

The performance of nanoceria-coated PADs for the analysis of H_2O_2 obtained from the pesticide assay was evaluated. H_2O_2 in the range of 0–100 mM was measured by dropping 5 μL of H_2O_2 onto nanoceria-coated PADs and the yellow color developed. The device was allowed to dry and the color intensity quantified by scanning the device using a desktop scanner (Canon CanoScan LiDE 110). The yellow color intensity was analyzed using ImageJ software (National Institute of Health, USA) using the previously described methods.[51]

Assay for OP pesticide analysis

The general procedure for the PAD-based OP pesticide analysis is shown in Scheme 1. The control where no pesticide was added was carried out by mixing 5 μL of 0.5 U mL^{-1} AChE with 5 μL DI water in a microcentrifuge tube followed by incubation for 15 min. Then, 5 μL of 5 U mL^{-1} ChOX and 5 μL of 3 mM acetylcholine were added to the tube. The solution was incubated for another 15 min. Finally, 20 μL of the mixture was dropped onto nanoceria-coated PAD and allowed to react for 20 min. After drying using a hair dryer, the device was scanned and the image was analyzed with ImageJ. For OP pesticide analysis, the assay was performed in a similar manner except that the pesticide samples were mixed with an AChE solution. The presence of an OP pesticide inhibited the AChE activity and produced less H_2O_2 resulting in a lower color intensity on nanoceria-coated PADs. In this work, two OP pesticides including methyl-paraoxon and chlorpyrifos-oxon were used as model OP pesticide standards.

Assay optimization

Studies of AChE, ChOX, acetylcholine and methanol concentrations as well as the reaction time were optimized on the PAD. All optimization experiments were performed at room temperature (28 ± 2 °C) without the addition of OP pesticides. For AChE optimization, different concentrations of AChE (0.1 to 15 U mL^{-1}, 5 μL) were mixed with acetylcholine (5 mM, 5 μL) and ChOX (20 U mL^{-1}, 5 μL) in a microcentrifuge tube and allowed to react for 15 min. The mixture was dropped onto nanoceria-coated paper-based devices and analyzed using the method described above. For ChOX and acetylcholine optimization, the experiments were carried out in a similar manner as AChE. For ChOX study, various concentrations of ChOX (0.1–20 U mL^{-1}) were evaluated where the concentration of acetylcholine and AChE were fixed at 5 mM and 1 U mL^{-1}, respectively. For acetylcholine optimization, acetylcholine in the range of 1–10 mM was studied while the concentrations of ChOX and AChE were kept at 5 U mL^{-1} and 1 U mL^{-1}, respectively. For studying the effect of methanol used to dissolve the pesticides, 0–10% v/v methanol was evaluated. Methanol solutions (0–10% v/v, 5 μL) and AChE (1 U mL^{-1}, 5 μL) were incubated in a microcentrifuge tube for 15 min followed by ChOX (5 U mL^{-1}, 5 μL) and acetylcholine (3 mM, 5 μL) and allowed

to react for 15 min prior to analysis. The mixture was pipetted onto the device and quantified by the method described above. To study the reaction time, the mixture of acetylcholine (3 mM, 5 μL), AChE (1 U mL^{-1}, 5 μL) and ChOX (5 U mL^{-1}, 5 μL) was allowed to react for 15, 20 or 30 min at room temperature. After reacting, the mixtures were dropped onto devices and analyzed using the procedure described above.

Sample preparation

To test the method accuracy, analysis of OP pesticides in spiked samples was performed using the PAD assay and a traditional LC-MS/MS method.[54] Cabbage and green mussel dried seafood samples spiked with MPO were employed for the study. Cabbage was taken from a supermarket and dried seafood samples were purchased from a local market in Chonburi province, Thailand. The samples were homogenized using a blender. 5 g of each sample was put into a 50 mL centrifuge tube and spiked with an OP pesticide standard MPO (100 μg mL^{-1}, 40 μL). The mixture was vortexed for 1 min and left in the dark for 15 min to create homogeneous samples. 20 mL of methanol was added to the mixture and then mixed for 30 min using a vortex mixer for extraction. The sample tubes were then centrifuged at 4000 rpm for 10 min. A 1.5 mL of supernatant was added into a 2 mL micro-centrifuge tube of dispersive SPE containing 25 mg of C18 and 150 mg $MgSO_4$ used for sample clean up and to remove the residual water. The mixture was vortexed for 30 min and centrifuged for 10 min at 4000 rpm and the supernatant was collected and dried in the hood. Finally, the extracted residue was re-dissolved in 1 mL of 4% methanol and 100% methanol for the analysis using the developed paper-based device assay and LC-MS/MS, respectively. The final concentration of MPO in the sample prior to analysis was 0.2 μg mL^{-1} in both samples. The traditional method, LC-MS/MS, was performed by Central Laboratory (Thailand) Co., Ltd Chachoengsao branch. The LC-MS/MS operating conditions were as follows: the HPLC WATERS, 269 model equipped with the MS/MS (Micromass, Quattro Ultima FS) was used with C18 2.0 × 150 mm, 3 μm column. Injection volume was 10 μL. Isocratic elution was used with a mobile phase mixture of 0.1% formic acid in H_2O and acetonitrile in the ratio of 40 : 60 with the flow rate of 0.2 mL min^{-1}. The mass selective detector was operated in electrospray ionization (ES) mode with the source and desolvation temperatures of 120 °C and 350 °C, respectively. The cone and desolvation gas flow were performed at 55–60 and 550–600 L h^{-1}, respectively.

Results and discussion

Organophosphate pesticides are well-known as the irreversible inhibitors of AChE in the central and peripheral nervous system of both insects and humans, causing a variety of toxicological effects on human health.[55] The assay principle used here is based on the enzymatic inhibition of AChE by the pesticides using nanoceria as a colorimetric agent as described in reactions (1) through (3). Normally, acetylcholine is catalyzed by AChE to form choline (reaction (1)) which is then oxidized by ChOX to generate H_2O_2 (reaction (2)).[56] The amount of H_2O_2 produced is measured colorimetrically by nanoceria when Ce^{3+} is oxidized to Ce^{4+} in the presence of H_2O_2 resulting in the color change from colorless to yellow.[47] While this is the most likely mechanism, other mechanisms for nanoceria color change upon the addition of H_2O_2 have been proposed.[47,57] The yellow intensity developed by the nanoceria is directly proportional to H_2O_2 yield. In the presence of OP pesticides, AChE activity is inhibited and hence produces less H_2O_2 yielding lower yellow color intensity for the nanoceria. The nanoceria as a colorimetric agent here was coated on paper-based device to allow for easy, inexpensive and portable detection of OP pesticides.

$$\text{Acetylcholine} + H_2O \xrightarrow{\text{AChE}} \text{Choline} + \text{Acetate} \quad (1)$$

$$\text{Choline} + O_2 \xrightarrow{\text{ChOX}} H_2O_2 \quad (2)$$

$$Ce_2O_3 + H_2O_2 \longrightarrow 2CeO_{2\ (ox)} + H_2O \quad (3)$$

Hydrogen peroxide analysis

We first evaluated the H_2O_2 analysis using nanoceria-coated PAD as it is a key for determining OP pesticides in this assay. The optimal nanoceria concentration was determined first. As the nanoceria concentration increased at constant H_2O_2 concentration, the yellow color intensity on the device increased until it saturated at 4% w/v (Fig. S2†). However, it was observed that the detection zone of the device became hydrophobic when 4% w/v or higher concentration of nanoceria was used due to a high degree of nanoceria aggregation. The hydrophobicity made it difficult for the sample to become wet throughout the detection zone in a reasonable amount of time.[46] As a result, 3% w/v nanoceria was chosen as the optimal concentration which is similar to the previous report.[49]

Under the optimal conditions, the nanoceria-coated PAD was used to analyze H_2O_2 response. The linearity, reproducibility, sensitivity and detection limit were determined. The color response on nanoceria-coated PAD depended on H_2O_2 concentration, as shown in Fig. 1A, where the color intensity increased at higher H_2O_2 concentration. The plot of mean gray intensity as a function of H_2O_2 concentration gave two linear ranges, one from 1–2.5 mM ($y = 4.7651x - 3.8942$, $R^2 = 0.998$) and a second from 3–12 mM ($y = 1.1584x + 5.6268$, $R^2 = 0.992$) (Fig. 1B). The sensitivity of the first linear portion of the curve is higher than that in the second portion as the color intensity becomes saturated at higher H_2O_2 concentration. Assay reproducibility in the range of 2.21–5.40% RSD ($n = 10$) was observed for H_2O_2 analysis across all tested concentrations indicating that the nanoceria-coated PAD provide reproducible H_2O_2 analysis. The H_2O_2 detection limit, defined as the concentration giving a signal three times higher than the standard

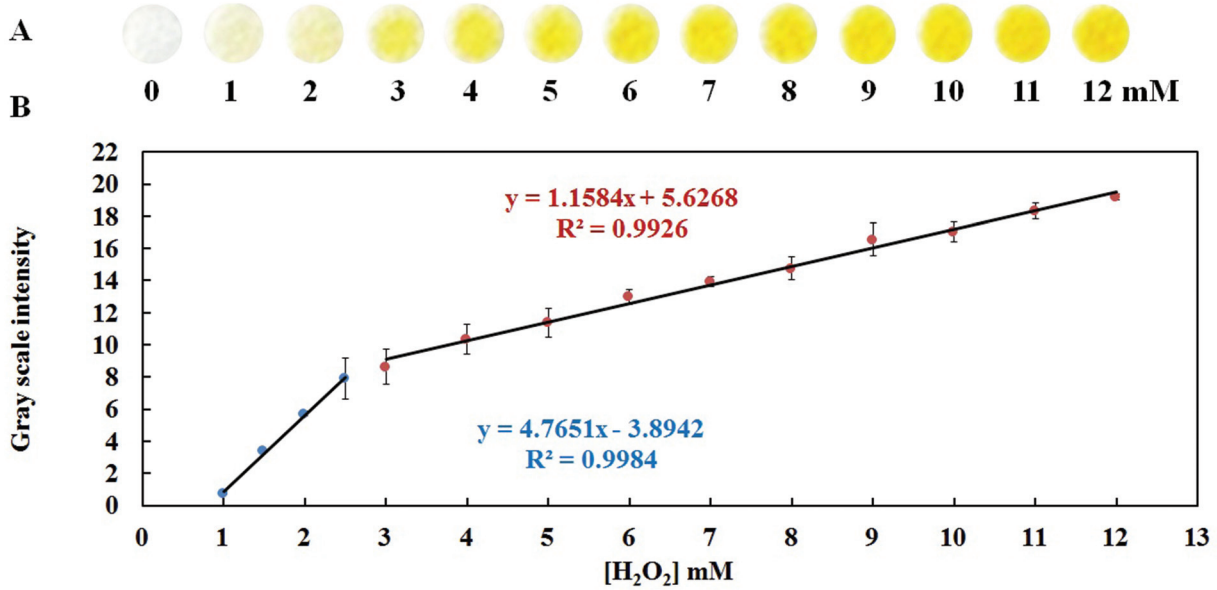


Fig. 1 The analysis of H_2O_2 using nanoceria-coated paper-based devices. (A) Typical devices from the analysis of different concentrations of H_2O_2. (B) Standard curve plotted of gray intensity as a function of H_2O_2 (n = 3) giving two linear portions where the first one has higher sensitivity than the second portion as the intensity became saturated at higher H_2O_2 concentrations.

deviation of the blank (DI water), was 0.5 mM. The results clearly demonstrate that the PAD can be effectively used to analyze H_2O_2 making it suitable for OP pesticide analysis. Moreover, when compared with the paper-based nanoceria assay reported previously, the device provides higher reproducibility and sensitivity for H_2O_2 analysis at lower H_2O_2 concentrations.[49]

Assay optimization for pesticide analysis

In this assay, pesticide analysis is based on the inhibition of AChE activity by OP pesticides. The optimal concentration of AChE was evaluated to determine sensitivity. A mixture of acetylcholine, AChE and ChOX without the addition of OP pesticides was performed to optimize the enzymatic assay. As shown in Fig. 2A, the yellow intensity increased as the AChE concentration increased from 0.1 to 1 U mL^{-1} as a result of increasing enzyme activity. However, when AChE concentration was higher than 1 U mL^{-1}, the color intensity became saturated. Therefore, the concentration of AChE in the range of 0.1–1 U mL^{-1} was considered as the optimal value to allow for sensitive-response analysis of OP pesticide. The result demonstrates that it is possible to measure AChE activity and thus OP pesticides that inhibit AChE activity in a dose–response manner.

The optimal concentration of acetylcholine and ChOX was evaluated next as they also affect assay sensitivity. The results showed that the intensity increased as acetylcholine and ChOX concentration increased and became saturated at 3 mM and 5 U mL^{-1} of acetylcholine and ChOX, respectively (Fig. 2B and C). Therefore, they were selected as optimal concentrations for further experiments. The reaction time was also studied in the range of 15–30 min. The results showed no difference in color intensity from 15 to 30 min as shown in Fig. S4.† Therefore, a reaction time of 15 min was selected for further experiments to provide minimized analysis time. Shorter reaction times were not tested.

The stock OP pesticide solution is normally prepared in organic solvents since the pesticides tested here are insoluble in water. However, it has been reported that organic solvents such as methanol can inhibit AChE activity.[58] Here, pesticide stock solutions were made in methanol and diluted with DI water. The amount of methanol in final pesticide solution that did not inhibit enzyme activity was measured. As shown in Fig. 2D, the yellow intensity decreased when methanol concentrations of ≥5% v/v was used indicating that the AChE was inhibited at these concentrations. As a result, methanol concentrations in the final pesticide solution were kept at 4% v/v to minimize impact on enzyme activity.

Pesticide analysis using nanoceria-coated paper devices

Once the optimized conditions were obtained, the analysis of model OP pesticides including methyl-paraoxon (MPO) and chlorpyrifos-oxon (CPO) were carried out for proof-of-concept. Dose–response curves for MPO and CPO at different starting concentrations of AChE are shown in Fig. 3A and B, respectively. For both pesticides, changing the initial concentration of AChE shifted the dose–response curves. At low AChE concentration (0.1 U mL^{-1}), the dose–response curve decreased quickly with increasing pesticide concentration, offering the highest sensitivity among the three AChE concentrations (0.1, 0.5 and 1 U mL^{-1}). However, the high sensitivities give small working ranges (0–0.1 μg mL^{-1} of MPO with R^2 = 0.988, 0–60 ng mL^{-1} of CPO with R^2 = 0.997). A higher AChE concentration

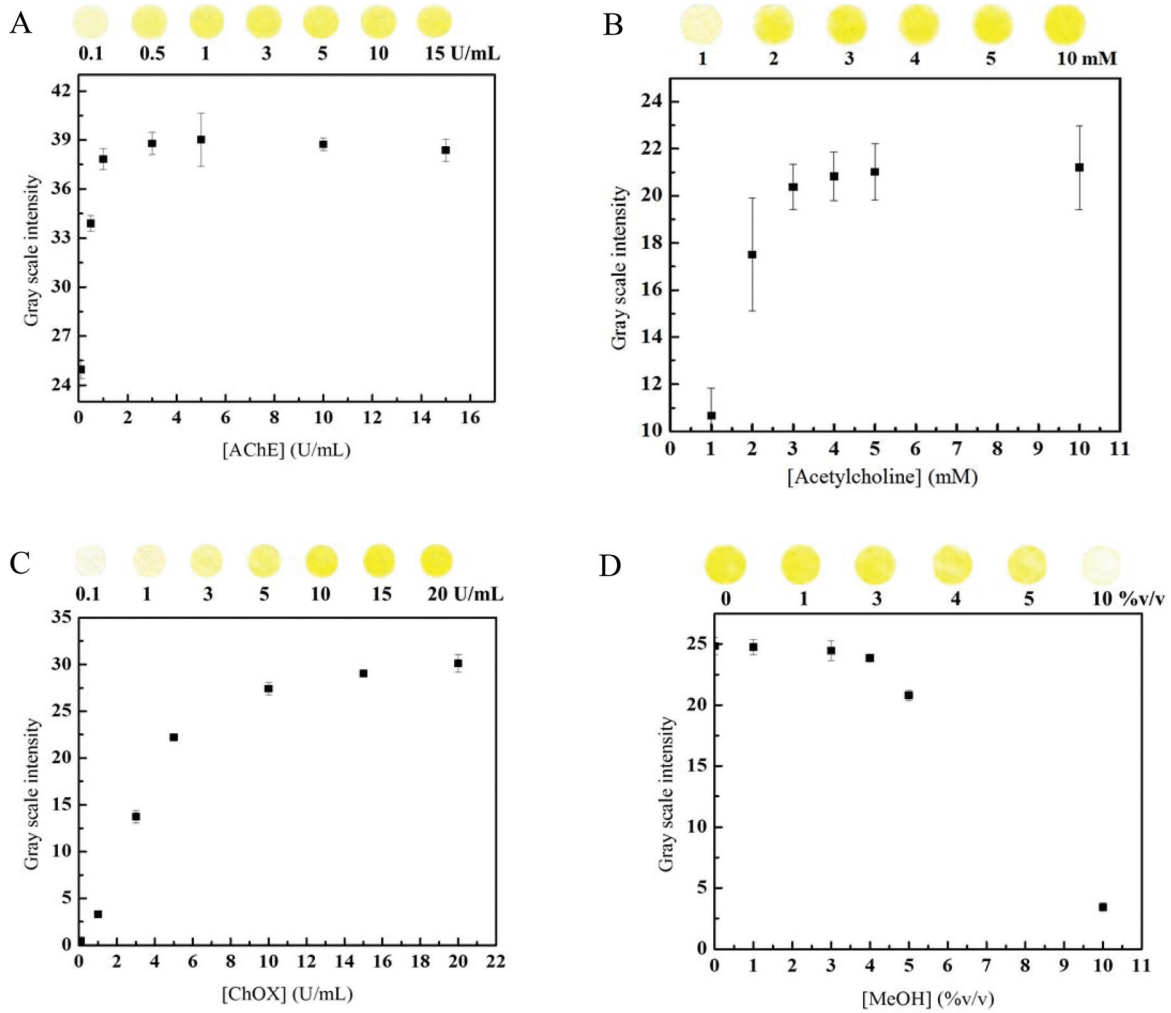


Fig. 2 The color responses of concentration of (A) AChE, (B) acetylcholine, (C) ChOX and (D) methanol. Photographs of actual responses are shown across the top of each plot.

(0.5 U mL^{-1}) gave lower sensitivities but wider working ranges (0–0.7 μg mL^{-1} of MPO with R^2 = 0.988, 0–80 ng mL^{-1} of CPO with R^2 = 0.984). Using the highest AChE concentration (1 U mL^{-1}), the lowest sensitivity was observed but the largest working range was obtained (0–0.9 μg mL^{-1} of MPO with R^2 = 0.993, 0–120 ng mL^{-1} of CPO with R^2 = 0.982). The differences in sensitivities for the pesticides are expected based on differences in their ability to inhibit AChE. For analysis of real world samples, this is still acceptable, however, because the system can be normalized to overall AChE inhibition. The limit of detection (LOD), defined as the pesticide concentration that causes a decrease in the signal three times the signal-to-noise ratio relative to the control, was observed to depend on the AChE concentration as well. A lower AChE concentration gave a lower LOD. For methyl-paraoxon, a detection limit of 18.3 ng mL^{-1} was obtained which is similar to the previously reported values.[34] For chlorpyrifos-oxon, the detection limit is 5.3 ng mL^{-1}, which is comparable to that reported with the performance of a portable biosensor prototype assay developed by Hildebrandt *et al.*[59] The detection limits of the CPO and MPO analysis provided by μPAD are low enough to appropriately determine both standards at the regulation levels. Under European Union (EU) regulation (EC) No 396/2005, the maximum residue levels (MRL) for MPO and chlorpyrifos (a CPO parent compound) in, for example, cabbage are 0.01 mg kg^{-1} and 1.0 mg kg^{-1}, respectively.[60] Analytical figures of merit for the analysis of the two model OP pesticides at different AChE concentrations are summarized in Table 1. The results demonstrate that the performance of the assay can be tuned by selecting an appropriate concentration of AChE. While the developed assay is able to detect the two standard OP pesticides at low concentrations with high sensitivities, it cannot identify specific OP pesticides in the samples. Mass spectrometry shown in the next section would be a secondary assay to identify OP compounds in the sample that is detected using primary screening enzymatic assay using the developed PAD.

The device cost has also been estimated for device fabrication and key reagents used for OP determination. The cost of

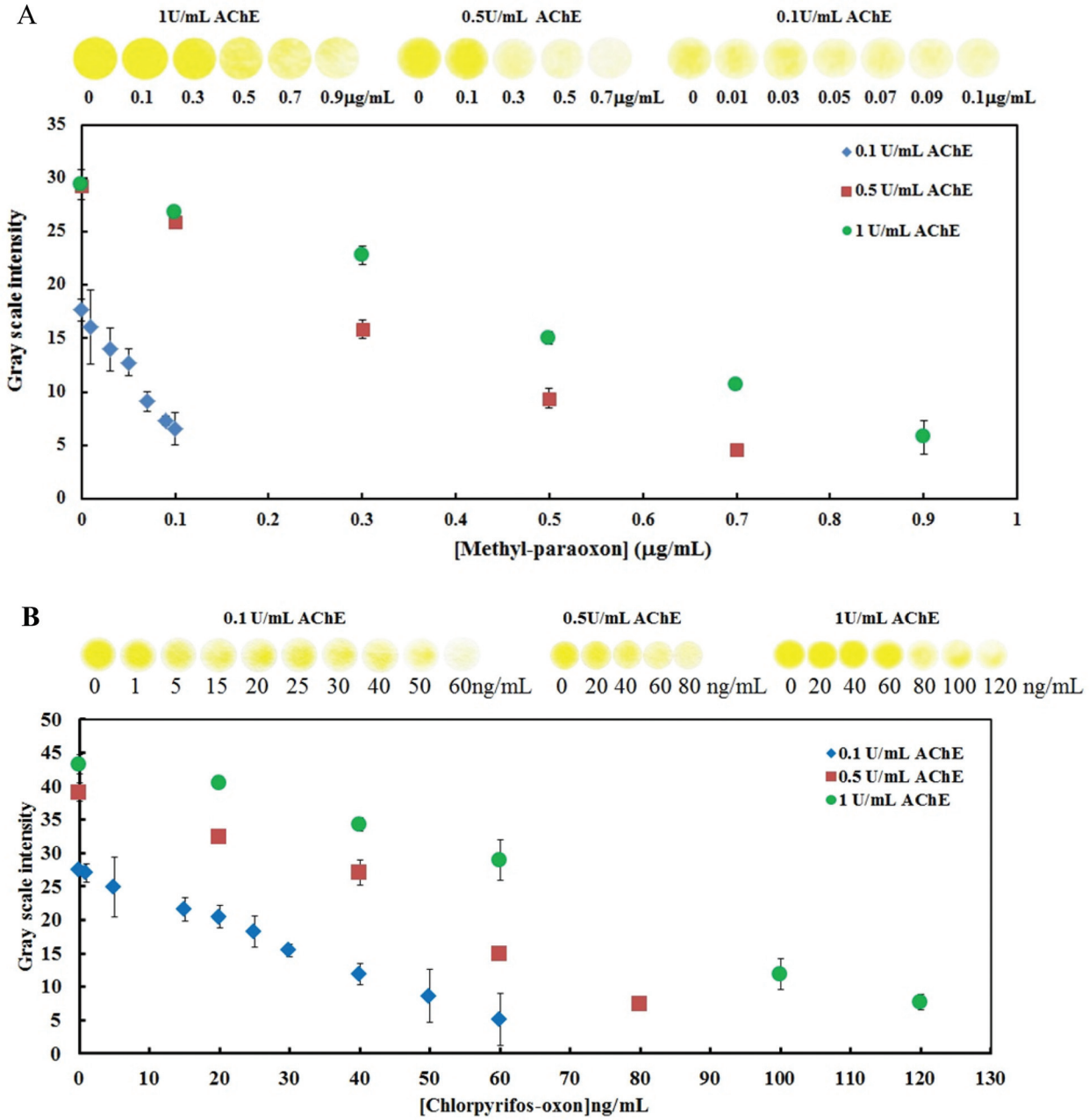


Fig. 3 Dose–response curves for the detection of OP pesticides using nanoceria-coated paper-based device with three concentrations of AChE. (A) methyl-paraoxon analysis and (B) chlorpyrifos-oxon analysis.

Table 1 Analytical figures of merit for OP pesticide analysis with three initial concentrations of AChE using nanoceria-coated paper-based device

OP pesticide	AChE (U mL^{-1})	LOD (ng mL^{-1})	Sensitivity	Linear range	R^2	%RSD (n = 7)
MPO	0.1	18	$-111\ (\mu g\ mL^{-1})^{-1}$	0–0.1 μg mL^{-1}	0.99	8.4–14
	0.5	31	$-36\ (\mu g\ mL^{-1})^{-1}$	0–0.7 μg mL^{-1}	0.99	2.1–21
	1	50	$-27\ (\mu g\ mL^{-1})^{-1}$	0–0.9 μg mL^{-1}	0.99	1.3–8.7
CPO	0.1	5.3	$-0.37\ (ng\ mL^{-1})^{-1}$	0–60 ng mL^{-1}	0.99	7.0–11
	0.5	9.8	$-0.40\ (ng\ mL^{-1})^{-1}$	0–80 ng mL^{-1}	0.98	3.3–13
	1	14	$-0.31\ (ng\ mL^{-1})^{-1}$	0–120 ng mL^{-1}	0.98	4.2–19

Table 2 Results of determination of methyl-paraoxon in spiked samples with nanoceria-coated paper-based device and LC-MS/MS method

Sample	Spiked MPO (μg mL^{-1})	Measured ± SD (n = 3)	
		This method	LC-MS/MS
Cabbage	0.00	ND	ND
	0.20	0.19 ± 0.02	0.22 ± 0.00
Green mussel	0.00	ND	ND
	0.20	0.19 ± 0.05	0.23 ± 0.01

ND: not detected.

the patterned paper fabrication was calculated and is shown in Table S1 (ESI†). Whatman# 4 (11 cm diameter) yields 125 detection zones. The cost per one detection zone was about $0.0011. Table S2† shows cost estimation of key chemicals used in this assay including enzymes and a substrate and the calculated cost was about $0.033. Therefore, the total cost for the determination of OP pesticides using this developed method was approximately $0.0341 per sample. The bulk expense comes from the enzymes used for one spot as 0.05 U and 0.025 U of AChE and ChOX were used, respectively. To the best of our knowledge, however, this assay required lowest amount of enzymes and reagents compared to that has been reported previously where up to 5 U of AChE was required for the lateral flow paper based sensor[4] and 3–5 U of AChE was needed for OP analysis using flow injection methods.[61,62]

Our device was also expected to be stable as the previously reported paper-based device that was coated with both enzymes and nanoceria was stable for 29 days and 79 days when stored at room temperature and in a refrigerator, respectively.[49] The device was also believed to be reusable when the absorbed peroxide species decomposed.[49]

Analysis of pesticides in real samples

Performance of the nanoceria-coated paper-based device was further evaluated for the analysis of MPO in spiked cabbage and dried green mussel samples. OP pesticides have been found in food crops including vegetables[63,64] and dried seafood products.[65,66] The validation procedure was performed by spiking both samples with 0.20 μg mL^{-1} MPO solutions. The extracted samples were quantified by both the PAD and LC-MS methods (Table 2). Using the PAD method, the measured MPO amounts of 0.19 ± 0.02 μg mL^{-1} (94.8% recovery) and 0.19 ± 0.05 μg mL^{-1} (95.1% recovery) were found in cabbage and dried green mussel, respectively. The two methods showed comparable results indicating that the paper-based method is able for the first time to make accurate measurements for the analysis of OP pesticide in complex matrix samples.

Conclusions

A nanoceria-coated paper-based device was developed for the detection of OP pesticides in foods. The determination of OP pesticides was based on enzyme inhibition as OP pesticide inhibits the AChE activity resulting in reduced H_2O_2 production for detection by nanoceria coated on the paper-based device. Various parameters including enzyme, substrate and organic solvent concentrations as well as reaction time were optimized to provide highly sensitive OP pesticide detection. Under optimal conditions, the analytical performance for measuring two model standard OP pesticides (MPO and CPO) were observed to depend on initial AChE concentration and gave detection limits as low as 18.3 ng mL^{-1} and 5.3 ng mL^{-1} for MPO and CPO, respectively. The PAD was validated against LC-MS/MS for the analysis of MPO spiked food samples. Similar measured MPO concentrations were obtained from both methods indicating a high degree of accuracy of the developed assay. While further efforts are underway to reduce sample preparation time, this new method is promising as an analytical tool for the rapid and low-cost screening of OP pesticides.

Acknowledgements

We would like to thank Dr. Karaked Tedsri for providing the synthesized nanoceria for initial study. This work was supported by (i) DPST Research Grant 013/2557 from the Institute for the Promotion of Teaching Science and Technology, Thailand, (ii) the Higher Education Research Promotion (HERP), Office of the Higher Education Commission, Ministry of Education, Thailand, and (iii) the center of excellence for innovation in chemistry (PERCH-CIC).

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