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A Label-Free Silicon Quantum Dots-Based Photoluminescence Sensor for Ultrasensitive Detection of Pesticides

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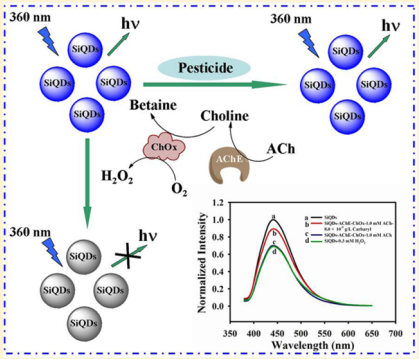
Supporting Information

ABSTRACT: Sensitive, rapid, and simple detection methods for the screening of extensively used organophosphorus pesticides and highly toxic nerve agents are in urgent demand. A novel label-free silicon quantum dots (SiQDs)-based sensor was designed for ultrasensitive detection of pesticides. This sensing strategy involves the reaction of acetylcholine chloride (ACh) with acetylcholinesterase (AChE) to form choline that is in turn catalytically oxidized by choline oxidase (ChOx) to produce betaine and H_2O_2 which can quench the photoluminescence (PL) of SiQDs. Upon the addition of pesticides, the activity of AChE is inhibited, leading to the decrease of the generated H_2O_2, and hence the PL of SiQDs increases. By measuring the increase in SiQDs PL, the inhibition efficiency of pesticide to AChE activity was evaluated. It was found that the inhibition efficiency was linearly dependent on the logarithm of the pesticides concentration. Consequently, pesticides, such as carbaryl, parathion, diazinon, and phorate, were determined with the SiQDs PL sensing method. The lowest detectable concentrations for carbaryl, parathion, diazinon, and phorate reached 7.25×10^{-9}, 3.25×10^{-8}, 6.76×10^{-8}, and 1.9×10^{-7} g/L, respectively, which were much lower than those previously reported. The detecting results of pesticide residues in food samples via this method agree well with those from high-performance liquid chromatography. The simple strategy reported here should be suitable for on-site pesticides detection, especially in combination with other portable platforms.



INTRODUCTION

Pesticides are widely used in agriculture all over the world, whereas they cause widespread contamination of air, water, soil, and agricultural products, eventually leading to long-term accumulation in ecosystems including humans.[1] The residues with highly toxic substances have been found to cause serious problems to human health even at very low concentrations.[2,3] According to the World Health Organization, 1.5 billion cases of diarrhea in children (leading to more than 3 million deaths) are caused by contaminated food and drinking water every year.[4] The high toxicity of pesticides is ascribed to their ability to irreversibly inhibit the activity of acetylcholinesterase (AChE) in the central and peripheral nervous system, resulting in the accumulation of the neurotransmitter acetylcholine in the body, which can lead to fatal consequences.[5] In order to avoid possible harm to humans and animals, it is of great importance to develop a highly sensitive and reliable assay for pesticide residues to improve food safety and to protect the ecosystem.

In the past decade, a number of analytical techniques have been devised to detect pesticides in food and water, such as fluorescent bioprobes,[6−9] colorimetric assay,[10,11] chromatography−mass spectrometry,[12−14] electrochemical analysis,[15−18] and enzyme-linked immunosorbent assay.[19−21] Although these methods have high selectivity and adequate sensitivity, most of these techniques suffer from the disadvantages of high costs, sophisticated instruments, and high requirements for testing staff possessing master professional skills and sample pretreatment, resulting in the fact that they are not suitable for on-site detection in most settings, especially in emergency cases.

Recently, novel nanomaterials have attracted great attention and have been intensively studied in biological analysis and detection because of their unique chemical or physical properties.[22,23] Currently, three kinds of approaches involving nanoparticles have been developed for the detection of pesticide exposure: (1) The first is the use of nanomaterial as enzyme carriers for loading a large amount of enzymes to enhance the detection signal.[24] Some of these approaches require a complex electrode modifying process or introduce additional substrates, which make a complex detection protocol. (2) The second is the use of nanomaterial as peroxidase- or oxidase-like activities catalysts to catalyze the oxidation of various substrates including 2, 2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt and 3,3,5,5-tetramethylbenzidine by enzyme-generated hydrogen

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peroxide (H_2O_2) for colorimetric or fluorescence detection of pesticides.[25,26] (3) The third is the use of nanomaterial as a direct signal source. For instance, Li's group developed a new approach for electrochemical quantification of organophosphorus pesticides using the stripping voltammetric signal of Cd^{2+} based on a CdTe QDs-gold nanoparticle composite.[27] Gao and colleagues reported the sensing of organophosphorus compounds based on a Mn-doped ZnSe QDs−enzyme−H_2O_2 fluorescence quenching system.[28] However, the heavy metal ion-containing QDs may suffer from intrinsic limitations such as potential toxicity, intrinsic blinking, and chemical instability.[29] Furthermore, the release of heavy metal ions may inhibit the activity of enzymes and is a potential environmental hazard, which limits the application of these QDs.[30] Therefore, it is also important to develop excellent nanomaterials for fabricating highly sensitive and selective biosensors.

Silicon quantum dots (SiQDs), as inert, nontoxic, abundant, and low-cost nanomaterials, have been demonstrated to be environmentally friendly photoluminescence probes and have attracted much interest. In comparison to other QDs, SiQDs have unique optical and electronic properties, especially favorable biocompatibility. These advantages enable them to play a great role in a variety of applications. To date, a few studies have reported that the modified SiQDs may be excellent candidates for biological imaging, but there were few studies on the sensitive detection of various analytes associated with enzyme-catalyzed events by using label-free SiQDs.

In this study, we report a SiQDs-based simple and highly sensitive sensor for pesticides. It is well-known that AChE can hydrolyze acetylcholine chloride (ACh) to choline, which can be oxidized to betaine with the concomitant generation of H_2O_2 in the presence of choline oxidase (ChOx).[31] The activity of AChE can be inhibited by pesticides.[32] Our previous work has been proved that the fluorescence of label-free SiQDs could be effectively quenched by enzyme-generated H_2O_2.[29] As shown in Figure 1, a novel strategy for pesticide detection was hence proposed. The sensing procedure is based on SiQDs fluorescence quenching by enzyme-generated H_2O_2 and the activity of AChE inhibited by pesticides. These proposed biosensors developed a new method for biomonitoring of trace pesticide exposures based on their inhibition effects on enzyme activity. This new type of biosensor does not require complex modification and enzyme immobilization, and the assay results can be read as soon as the probe-sample incubation is completed.

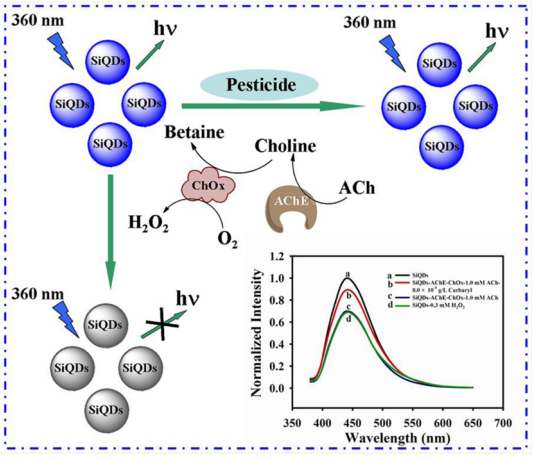

Figure 1. Schematic illustration of the measuring principle of the pesticide biosensor. Inset: PL spectra of SiQDs mixtures, (a) only SiQDs and buffer, (b) SiQDs + 2 U/mL AChE + 0.6 U/mL ChOx + 1.0 mM ACh + 8.0×10^{-5} g/L carbaryl, (c) SiQDs + 2 U/mL AChE + 0.6 U/mL ChOx + 1.0 mM ACh, and (d) SiQDs + 0.3 mM H_2O_2, respectively.

EXPERIMENTAL SECTION

Materials and Instrumentation. All chemicals from commercial sources were of analytical grade and used without further purification. AChE (from *Electrophorus electricus*), ChOx (from *Alcaligenes* sp.), and ACh were obtained from Sigma-Aldrich. Carbaryl, parathion, diazinon, and phorate were from Huaerbo Chemical Reagent Co. Silicon wafers (phosphorus-doped (p-type), 8Ω resistivity) and phosphomolybdic acid (POM) were purchased from Sigma-Aldrich. Anhydrous ethanol (analytical grade), hydrofluoric acid (HF), and H_2O_2 were purchased from Shanghai Chemical Reagent. PBS buffer (pH 8.0, 5 mM sodium phosphate) and Milli-Q ultrapure water (Millipore, ≥ 18 MΩ cm) were used throughout.

SiQDs synthesis was conducted on a CHI660A electrochemical workstation (CHI Instrument Inc., USA). UV−vis spectra were recorded with a UV2450 spectrophotometer (Shimadzu). The fluorescence spectra were collected on a Hitachi F-4500 fluorescence spectrophotometer operating at the excitation wavelength of 360 nm, with both excitation and emission slit widths of 10 nm. The fluorescence intensities at 440 nm were measured. Transmission electron microscopy (TEM) images were obtained by using a JEOL-1230 model. Fourier transform infrared (FTIR) spectra were collected on a Nicolet Nexus 670 FTIR spectrometer (Nicolet Instrument Co., U.S.A.). High-performance liquid chromatography (HPLC; Agilent 1200, U.S.A.) analysis was performed on a Zorbax SB-C_{18} column (150 mm × 4.6 mm i.d., 4 μm). A mixture of methanol−water (80:20 v/v) was used as the isocratic mobile phase to deliver the sample containing carbaryl with a flow rate of 0.8 mL/min at 25 °C. Apple, tomato, and cucumber solutions were prepared with a juice extractor (Philips, HR2010, 350 W) bought from a supermarket.

Synthesis of SiQDs. Photoluminescence SiQDs were synthesized by the POM-assisted electrochemical etching of bulk Si. Briefly, 0.015 g of POM was dissolved in 35 mL of anhydrous ethanol, then 35 mL of HF was added under stirring. Until the solution became transparent, a silicon wafer and carbon rod (length of 5 cm) were immerged, where the silicon wafer worked as the working electrode and the carbon rod served as the reference and counter electrode. An amperometric i−t curve was selected. The initial potential was about 7−10 V, and the current density was kept in the range of 4−10 mA cm^{-2}. After etching for about 2 h, large amounts of SiQDs formed on the surface of the silicon wafer. Without complicated centrifugation and filtration, just ultrasonication fracturing of the etched silicon wafer in absolute ethanol occurred, and then SiQDs with excellent fluorescence were obtained. The prepared SiQDs were characterized using fluorescence spectroscopy and TEM.

Experimental Procedure for the Sensitive Assay of Pesticides. Pesticides inhibit the activity of the AChE enzyme and induce a decrease in the PL quenching efficiency of SiQDs by enzyme-generated H_2O_2. One of the most commonly used pesticides, carbaryl, was investigated as the representative model pesticide. Carbaryl was determined by measuring the decrease in quenching efficiency of the SiQDs PL in the

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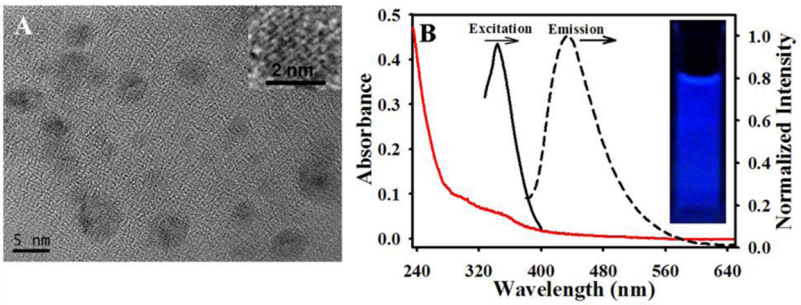

Figure 2. (A) Transmission electron microscopy image of SiQDs. The inset shows the high-resolution transmission electron microscopy image of SiQDs. (B) UV−vis absorption and excitation spectra (solid line) and emission spectra (dot line) of SiQDs. The inset shows the photo of SiQDs illuminated by UV light of 365 nm.

presence of the AChE and ChOx. AChE (2 U/mL) was first incubated with various concentrations of carbaryl in 50 μL of PBS (5 mM, pH 8.0) for 10 min at room temperature. The resultant solution was then added to the assay solution prepared by suspending the SiQDs in 450 μL of PBS (5 mM, pH 8.0) which contained ChOx (0.6 U/mL) and ACh (1 mM). The mixed solution was incubated for another 15 min in the dark at 40 °C. The PL intensity of SiQDs at 440 nm was collected in the final reaction solution.

Analysis of Carbaryl Residues in Food Sample. Apple, tomato, and cucumber were chosen as the sample matrix to evaluate the carbaryl residue levels in the real application tests of this pesticide assay. A standard carbaryl solution (0.1 g/L) was sprayed onto skins of the food samples by an atomizer, and the carbaryl residues in aliquots of the samples were collected every other day over the course of 10 days. The samples were first chopped, and the edible parts of the samples were crushed into a homogenate by a juice extractor. A total of 25 g of each homogenate was mixed with 50 mL of acetonitrile, and the resulting mixture was filtered through a 0.22 μm membrane to remove the insoluble materials. The obtained filtrate was dried on a water bath, and the remaining solid substance was mixed with methanol to a final volume of 5 mL. Subsequently, 30 μL of the final carbaryl residue solution was added to 1 mL of the SiQDs, AChE, ChOx, and ACh mixture solution (SiQDs−AChE−ChOx−ACh) and incubated in a water bath at 40 °C for 15 min. Then, the mixture was taken out from the water bath and allowed to cool to room temperature for 5 min for PL measurements at an excitation wavelength of 360 nm.

RESULTS AND DISCUSSION

Characterization of the SiQDs. The morphology and optical properties of SiQDs were characterized, and the results are shown in Figure 2. TEM (Figure 2A) and the high resolution transmission electron microscopy (HRTEM; inset of Figure 2A) images show that the SiQDs were mostly spherical dots, and the size is uniform, with an average diameter of about 5 nm. The absorption, excitation, and emission spectra of SiQDs in aqueous solution are presented in Figure 2B. The absorbance below 290 nm is a characteristic absorbance of SiQDs.[33] A narrow emission spectrum range from 400 to 500 nm was observed with the SiQDs solution, which illustrates that the size of the SiQDs is uniform.[34] As shown in the inset of Figure 2B, the SiQDs emitted bright blue light under UV light excitation. The prepared SiQDs are terminated with Si−H bonds, as revealed by FTIR measurement (Figure S1, Supporting Information). The strong stretching modes of monohydrides Si−H bonds occurred at around 900 cm^{-1}, and coupled H−Si−Si−H stretch bonds occurred at 2100 cm^{-1}.[35] The PL quantum yield of SiQDs was up to ~9.6% according to the Williams method[36] (the detailed process shown in the Supporting Information). Compared with other reported methods, the obtained SiQDs have very high quantum yields.[37,38] Upon further investigation, we found that the PL intensity of SiQDs was not affected by temperature (Figure S2A, Supporting Information) and reached a maximum when the pH of SiQDs in solution was 7.5−8.0 (Figure S2B, Supporting Information). Moreover, the SiQDs diluted with PBS (pH 8.0) possessed excellent photostability over 6 h in air under ambient conditions without any protection (Figure S2C, Supporting Information).

Quenching Effect of H_2O_2 on the PL Emission of Label-Free SiQDs. The proposed sensing strategy of pesticides is based on label-free SiQDs and the enzyme reaction of ACh. The basic principle of the SiQDs-based pesticide sensor includes the hydrolysis of ACh catalyzed by AChE, the oxidation of choline in the presence of ChOx, the inhibition of enzyme activity by pesticides, and PL quenching of SiQDs by the enzyme-generated H_2O_2.

$$\mathrm{ACh} + \mathrm{H_2O} \xrightarrow{\mathrm{AChE}} \mathrm{Choline} \quad (1)$$

$$\mathrm{Choline} + \mathrm{O_2} \xrightarrow{\mathrm{ChOx}} \mathrm{H_2O_2} + \mathrm{Betaine} \quad (2)$$

To determine the concentration of pesticides, the correlations of the inhibition effects of pesticides, the enzyme-generated H_2O_2, and the PL quenching effect of SiQDs should be established. Herein, we first investigated the influence of the H_2O_2 or AChE−ChOx−ACh in the absence and presence of carbaryl on the PL emission of the SiQDs. As can be seen from Figure 1, the PL intensity of the label-free SiQDs solution evidently decreased after incubation with H_2O_2 (curve d) or AChE−ChOx−ACh (curve c). While the label-free SiQDs were individually incubated with ChOx, AChE, ChOx and AChE, ACh, or carbaryl under the same conditions, almost no change was observed in SiQDs PL (Figure S3, Supporting Information). In addition, when the AChE−ChOx−ACh was pretreated with carbaryl before it was incubated with the label-free SiQDs, the SiQDs PL decrease was effectively prevented. In order to determine the effect of oxygen dissolved, time-dependent PL spectra of SiQDs−AChE−ChOx system in the saturated oxygen atmosphere were investigated (Figure S4, Supporting Information). The results indicated that the SiQDs−AChE−ChOx system possessed excellent photostability over 60 min in the saturated oxygen atmosphere and implied that the oxygen dissolved in the SiQDs−AChE−ChOx system

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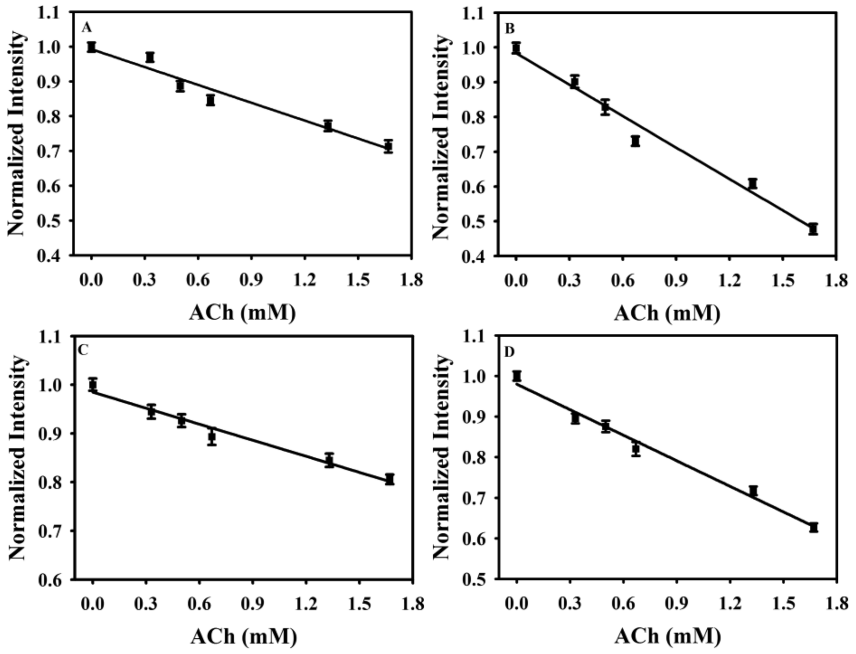

Figure 3. SiQDs PL intensity vs ACh concentration. The concentrations of AChE and ChOx are 2 U/mL and 2 U/mL (A), 2 U/mL and 0.6 U/mL (B), 5 U/mL and 0.3 U/mL (C), and 0.3 U/mL and 2 U/mL (D), respectively.

has no influence of the SiQDs PL. Figure S5 (Supporting Information) showed the PL intensities of SiQDs solution after treatment with different amounts of H_2O_2. It was observed that the PL intensity of SiQDs decreased gradually with the increasing of H_2O_2. These facts assuredly testified to the quenching effect of the enzyme-generated H_2O_2 on SiQDs PL and the inhibition effect of pesticides on the enzyme activity. The PL quenching mechanism of SiQDs by H_2O_2 (from catalytic oxidation of choline generated from the reaction of ACh with AChE) can be explained as follows: Since H_2O_2 contains the active oxygen species, it can diffuse across the surface of the SiQDs and capture the electrons at the conduction bands of SiQDs, and subsequently the radiative recombination of the photoinduced electrons and holes is inhibited.[39] Therefore, H_2O_2 was capable of capturing the electrons of SiQDs as well as the quenching of the SiQDs PL.[34] The quenching process was also supported by TEM analysis (Figure S6, Supporting Information). The SiQDs remained well dispersed and highly uniform in size after the treatment of AChE−ChOx−ACh. But, the lattice fringe became blurred (the HRTEM image inset in Figure S6, Supporting Information), suggesting that the reaction of H_2O_2 and SiQDs occurred. Upon the above observations, we deduce that the inhibition efficiency of pesticides on the activity of the enzyme could be correlated with the quenching extent of the SiQDs PL. By measuring the SiQDs PL of the carefully designed test system, the quantification of pesticides can be achieved.

Influence of SiQDs on Enzyme Activity. It is important to evaluate the influence of the label-free SiQDs on the activity of the used enzyme for pesticide detection following the proposed sensing strategy. The Ministry of Health Determination Standard of the AChE and ChOx (WS/T66-1996) has been selected for monitoring the enzyme activity changes in the presence and absence of SiQDs.[40] The absorbance spectra and color changes of variable concentrations of ACh upon the interaction with AChE, ChOx, and alkaline hydroxylamine are shown in Figure S7 (Supporting Information). There are no significant differences between the results in the presence (Figure S7A and S7C, Supporting Information) and absence (Figure S7B and S7D, Supporting Information) of SiQDs, indicating that the SiQDs have no effect on enzyme activity.

Optimization of the Detection Conditions. To sensitively detect pesticides, figuring out the influences of the incubation time, temperature, and pH value on the SiQDs PL is necessary. As shown in Figure S8A (Supporting Information), the value of the quenching efficiency ($(I_0 - I)/I_0$, I_0 and I refer to the PL intensity of SiQDs−AChE−ChOx system in the absence and presence of ACh) reached a maximum when the temperature reached 40 °C. While verifying the pH value of the system in the presence of 1.0 mM ACh (Figure S8B, Supporting Information), the quenching efficiency reached a maximum at pH 8.0. Figure S8C (Supporting Information) shows that the quenching efficiency sustained a stable value when the incubation time reached 15 min. On the basis of those observations, the analyte systems were incubated at 40 °C for 15 min under a pH of 8.0 before fluorescence measurement in further experiments.

The concentrations of AChE and ChOx would have influences on the sensitivity of the proposed sensing strategy for pesticide detection, because the concentrations of AChE and ChOx have an effect on the enzyme generated H_2O_2 in the two-enzyme coupled reaction system and consequently affect the SiQDs PL intensity. This conjecture was experimentally confirmed by the results presented in Figure S9 (Supporting Information). Performing linear regression analysis between the SiQDs PL intensity and the ACh concentration presented in Figure S9(A−D) (Supporting Information), four linear equations were obtained, which could be expressed as (A) $Y = -0.1712X + 0.9927$, (B) $Y = -0.3016X + 0.9836$, (C) $Y = -0.1098X + 0.9849$, and (D) $Y = -0.2100X + 0.9801$, respectively, where Y refers to the PL intensity and X represents the concentrations of ACh. Figure 3 shows linear regression results, the PL intensity of the SiQDs−AChE−ChOx system decreased with the increasing concentration of ACh in all tested

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cases. The absolute value of the slope (|k|) of the four lines is ordered as $|k_B| > |k_D| > |k_A| > |k_C|$, which indicates that the highest quenching effect was reached when the concentrations of AChE and ChOx were 2 U/mL and 0.6 U/mL, respectively. Therefore, we use 2 U/mL AChE and 0.6 U/mL ChOx in further experiments.

Similar to the previous reports,[28,41] the absolute quenching rate is related to the Stern−Volmer constant. From Figure 5A, the linear Stern−Volmer plots (PL intensity vs incubation time) are obtained with K_{10} values (which were calculated as the slope of the plot of the PL intensity change vs reaction time for the first seven data points (the first 10 min)). K_{10} values were calculated via eq 3, where I_0 and I_{10} are the PL intensity recorded at 440 nm before and after the addition of ACh (first 10 min), respectively. The inhibition efficiency (IE) values were calculated via eq 4, where $K_{10without}$ and K_{10with} are the absolute quenching rates without inhibition and with inhibition at a certain concentration of pesticides, respectively. On the basis of the inhibition efficiency, the optimized concentration of ACh was tested as well. As shown in Figure 4, the inhibition efficiency increased with the increasing concentration of ACh from 0 to 1.0 mM, and then the response shows a slight decrease when the concentration of ACh is higher than 1.0 mM, which could be attributed to the phenomenon known as substrate inhibition.[42] When the ACh concentration was higher, the high concentration of enzymes was not conducive to the quenching effect of H_2O_2, because the excess ACh covered on the surface of SiQDs would inhibit the quenching effect. Therefore, in the present study, an ACh concentration of 1.0 mM was chosen for pesticide determination.

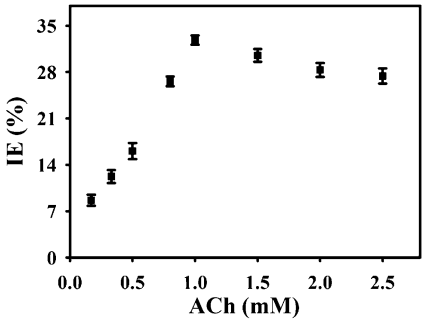

Figure 4. Inhibition efficiencies of carbaryl on enzyme vs ACh concentrations. The AChE, ChOx, and carbaryl concentrations are 2 U/mL, 0.6 U/mL, and 8.0×10^{-7} g/L, respectively.

$$K_{10} = \frac{(I_0 - I_{10})}{10} \tag{3}$$

$$IE = \frac{(K_{10without} - K_{10with})}{K_{10without}} \times 100 \tag{4}$$

Sensitive Assay for Pesticides. Figure 5A shows the time-dependent PL intensity of SiQDs upon treatment of the SiQDs−AChE−ChOx system in the presence of 1.0 mM ACh with variable concentrations of carbaryl. As the time was prolonged, the quenching effect of the QDs was reinforced. The result indicated that the SiQDs had been quenched by H_2O_2 which generated during the AChE and ChOx catalytic reactions. Figure 5B shows the relationship between inhibition efficiency and the carbaryl concentration, which inferred that the inhibition efficiency was linearly dependent on the logarithm of the carbaryl concentration in the concentration range of 7.49×10^{-9} g/L to 7.49×10^{-4} g/L, and the corresponding linear function is IE (inhibition efficiency, %) = 15.4552 log[carbaryl] (g/L) + 130.8024 with a correlation coeffcient of $R^2 = 0.9903$. The limit of detection (LOD; defined as the concentration of inhibitor required to achieve 5% inhibition[43]) was 7.25×10^{-9} g/L, which is much lower than the maximum residue limits (MRLs) as reported in the European Union pesticides database (MRL is 0.05 ppm for carbaryl)[44] as well as that of the United States (MRL is 0.02 ppm for carbaryl).[45]

To demonstrate that this assay method was effective not only for carbaryl but also for other pesticides, we investigated the sensitivity of the sensing platform for three other conventional pesticides: parathion, diazinon, and phorate. The experimental procedures were the same as that for carbaryl. Figure S10A,C,E show incubation time dependence of the PL intensity of the SiQDs−AChE−ChOx system vs variable concentrations of diazinon (A), parathion (C). and phorate (E). They had a similar trend to that of carbaryl. The lowest detectable concentrations for parathion, diazinon, and phorate are 3.25 $\times 10^{-8}$, 6.76×10^{-8}, and 1.9×10^{-7} g/L, respectively, which is much lower than those previously reported (Table S1).

Inhibition Efficiencies of the Pesticides. As different pesticides would have different inhibition efficiencies with respect to AChE, the inhibition efficiency of four pesticides at the concentration of 8.0×10^{-7} g/L was also investigated and is

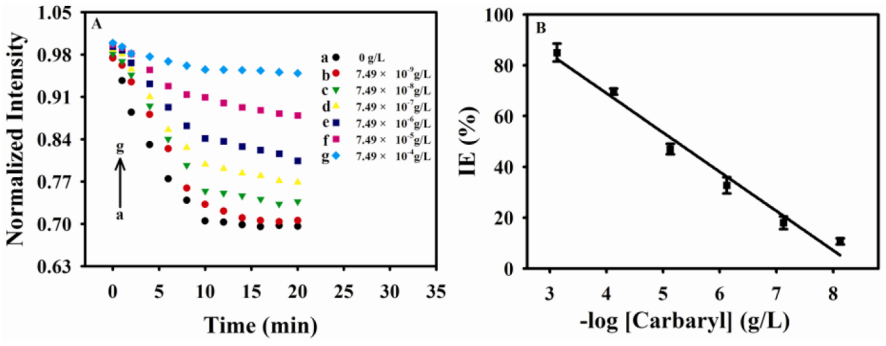

Figure 5. (A) Incubation time dependence of the PL intensity of the SiQDs−AChE−ChOx system in the presence of 1.0 mM ACh and a variable concentration of carbaryl. Carbaryl concentration (a−g): 0, 7.49×10^{-9} g/L, 7.49×10^{-8} g/L, 7.49×10^{-7} g/L, 7.49×10^{-6} g/L, 7.49×10^{-5} g/L, and 7.49×10^{-4} g/L. (B) Inhibition efficiency vs the logarithm of carbaryl concentration. All measurements were performed in PBS, pH = 8.0. The concentrations of AChE and ChOx are 2 U/mL and 0.6 U/mL, respectively.

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shown in Figure S7 (Supporting Information). It was observed that the IE values of carbaryl, diazinon, and parathion were considerably higher than that of phorate (12.48%), which were 32.47%, 30.85%, and 26.48%, respectively. The differences in the inhibition efficiency may result from the disparities in the molecular characteristics of the pesticides and agreed with the quantum chemistry simulations (Gaussian 03).[46] The changes in the conformation of AChE on the surface of SiQDs may also be responsible to these observations. Further studies on how the molecular characteristics of pesticides and conformational changes affect the inhibition efficiency are demanded in future work.

Anti-Interference Capability of the Sensor. It is essential to estimate the anti-interference capability of the sensor from the coexisting species in the analyte. Because this work is aimed at the development of a new sensing strategy for detecting pesticide residues, the common existing substances in food samples, such as glucide, metal ions, organic acids, and amine acids, were studied as the controls. As shown in Figure 6,

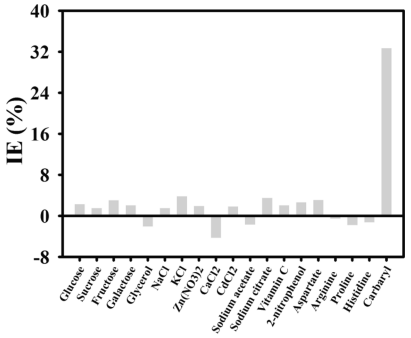

Figure 6. Effects of possible interference species in analytes (1.0×10^{-3} g/L) on the inhibition efficiency of carbaryl (8.0×10^{-7} g/L) in the SiQDs–AChE–ChOx system.

glucose, sucrose, fructose, galactose, and glycerol exhibited little influence on carbaryl detection even at concentrations 10^3 times higher than that of carbaryl. Typical metal ions, organic acids, and amine acids almost showed no interference in carbaryl determination. These results clearly demonstrate that the SiQDs based PL sensor is of high specificity for pesticide detection and has a strong capability to resist interference.

Determination of Carbaryl Residues in the Food Samples. The excellent specificity and high sensitivity of the sensor suggest that the developed method might be directly applied for detecting pesticide residues in real samples. Therefore, we further investigated whether the sensing strategy described here could be utilized to monitor the residues of pesticides in fruit and vegetable samples such as apples, tomatoes, and cucumbers. The carbaryl residues in aliquots of the food samples were collected every other day over the course of 10 days. The concentration of carbaryl residue was determined using a novel sensing strtegy based on a SiQDs–AChE–ChOx–ACh system and was also analyzed with HPLC to test the accuracy of this method. The carbaryl concentrations in food samples detected by the proposed assay and HPLC are shown in Figure 7. The results obtained by using this assay coincided well with those obtained by using HPLC, indicating that the proposed method can be successfully applied for detecting pesticide residues in real samples.

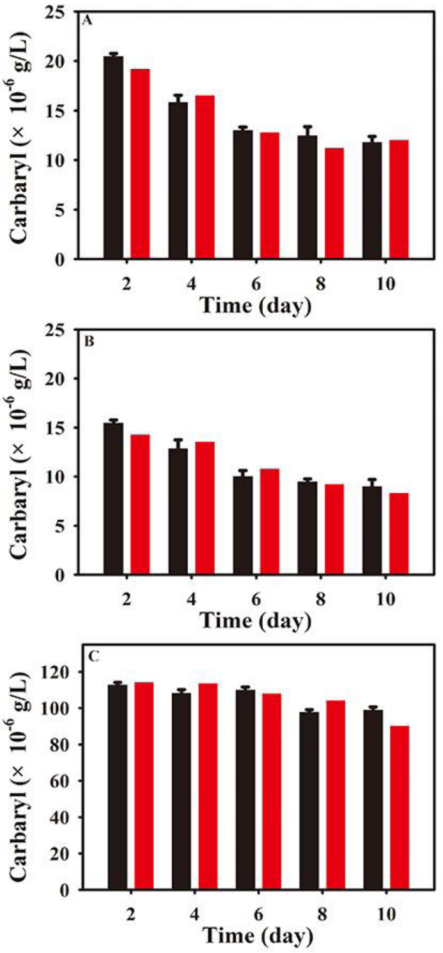

Figure 7. Analytical results of carbaryl in apple (A), tomato (B), and cucumber (C) samples obtained by using the present SiQDs–AChE–ChOx based method (black) and by using HPLC (red). All measurements were performed in PBS, pH = 8.0, thermostated at 40 °C.

CONCLUSIONS

In conclusion, we have developed a novel label-free SiQDs-based sensor for the detection of pesticides. The two-enzyme (AChE and ChOx) coupled reaction system is involved in this new developed sensing strategy. It has been experimentally demonstrated that the label-free SiQDs have no effect on the enzyme activity, and the enzyme generated H_2O_2 can effectively quench the PL of SiQDs. As the PL of SiQDs is very sensitive to H_2O_2, the measurements of SiQDs PL in the designed system could be used to evaluate the amount of the enzyme generated H_2O_2, which reflects the activity level of the enzymes. And it is found that the inhibitory effect of pesticides on the enzyme activity (termed the inhibition efficiency and evaluated by measuring the SiQDs PL) was linearly dependent on the logarithm of the pesticide concentration. The lowest detectable carbaryl concentration in this newly developed sensing strategy is much lower than the maximum residue limits of the European Union and U.S. Department of Agriculture, and the accuracy of this assay in real sample analysis is comparable to the traditional HPLC method, which indicates that the sensing strategy is of prospective application for pesticide residue detection in foodstuffs. Because this assay has the significant advantage of being simple, rapid, highly sensitive, and capable of anti-interference in pesticide detection, we believe this assay

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can be useful in monitoring pesticides in emergency cases. In addition, the research provides a new approach to further design novel nanosensors based on the assembly of SiQDs with other redox enzymes and a new method for the detection of other molecules.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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