

Microchip-Based Organophosphorus Detection Using Bienzyme Bioelectrocatalysis

Yong Duk Han, Chi Yong Jeong, Jun Hee Lee, Dae-Sik Lee¹, and Hyun C. Yoon*

Department of Molecular Science and Technology, Ajou University, Suwon, Gyeonggi 443-749, Republic of Korea

¹BT Convergence Research Laboratory, ETRI, Daejeon 305-700, Republic of Korea

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We have developed a microsystem for the detection of organophosphorus (OP) compounds using acetylcholine esterase (AChE) and choline oxidase (ChOx) bienzyme bioelectrocatalysis. Because AChE is irreversibly inhibited by OP pesticides, the change in AChE activity with OP treatment can be traced to determine OP concentration. Polymer-associated ChOx immobilization on the working electrode surface and magnetic microparticle (MP)-assisted AChE deposition methods were employed to create an AChE–ChOx bienzyme-modified biosensing system. ChOx was immobilized on the micropatterned electrodes using poly(L-lysine), glutaraldehyde, and amine-rich interfacial surface. AChE was immobilized on the MP surface via Schiff's base formation, and the enzyme-modified MPs were deposited on the working electrode using a magnet under the microfluidic channel. The bioelectrocatalytic reaction between AChE–ChOx bienzyme cascade and the ferrocenyl electron shuttle was successfully used to detect OP with the developed microchip. This provides a self-contained and relatively easy method for OP detection. It requires minimal time and a small sample size, and has potential analytic applications in pesticides and chemical warfare agents.

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1. Introduction

Organophosphorus (OP) compounds are used as pesticides, insecticides and chemical warfare agents.^{1–7} The critical toxicity of OP compounds to the human nerve system and their widespread use in modern agricultural practices has caused public concern, making the detection of OP compounds an important subject. The acetylcholine esterase (AChE) inhibition mechanism has been widely used to determine if a compound contains OP. Conventionally, acetylthiocholine (ATCh) was used as an AChE substrate for electrochemical OP biosensors.^{8–12} In the method using ATCh as a substrate, AChE decomposes ATCh into acetic acid and thiocholine (TCh) which is an electroactive species. But TCh, the product of AChE enzyme reaction, has a significant disadvantage in electrochemical analysis because it requires a high overpotential for electrooxidation and signal generation. In order to overcome this, ATCh was replaced by acetylcholine (ACh) as the AChE substrate and choline oxidase (ChOx) was employed to generate the electrochemical. In this system, ACh is decomposed into choline (Ch) and acetic acid by AChE reaction, and choline is then used as ChOx substrate, which generates an amplified electrochemical signal based on its electrocatalytic reaction (Fig. 1). The sequential AChE–ChOx bienzyme reaction could be turned into an electrochemical signal using electroactive mediators such as ferrocenemethanol.^{13–17} In our previous study, we have developed an OP biosensing system using this AChE–ChOx bioelectrocatalytic reaction successfully.¹⁸ However, the separated enzyme reaction steps in our previous version of OP biosensor was not proper to use in point-of-care-testing (POCT) application. To overcome this, in this work, we employed two different methods to arrange AChE and ChOx molecules on a single microdevice those are allowing efficient AChE–ChOx sequential bienzyme reactions. First, ChOx was directly immobilized on the electrode surface via crosslinking using a biocompatible polymer, amine-reactive linker, and a self-assembled interfacial layer on the electrode surface. Second, AChE was tagged to magnetic particles (MPs) with amine reactive groups (aldehyde) on their surface. After the

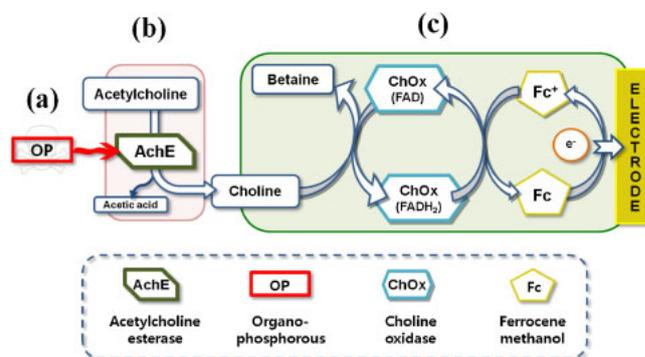


Fig. 1. (Color online) Schematic illustration of electrochemical OP detection. (a) AChE inhibition using the OP treatment. (b) Induction of AChE enzyme reaction (conversion of acetylcholine into choline). (c) Electrocatalytic reaction using ChOx and ferrocenemethanol.

enzyme modification, a magnet was used to deposit AChE-tagged MPs on the electrode surface based on the role of each enzyme. ChOx, the signaling redox enzyme, was tightly immobilized on the electrode surface and AChE-tagged MPs deposited in proximity to this ChOx electrode surface in order to obtain a strong electrochemical signal from this bienzyme reaction.^{18–23} Employing an MP as an immobilization matrix enlarges the reactive surface area of AChE. This allows for increased loading of AChE, enhancement of the AChE inhibition reaction by OP, and generation of a scalable signal. After OP detection, deposited and inhibited AChE-MPs can be removed from the ChOx electrode by eliminating magnetism. The resulting MP-removed ChOx electrode can be used to another OP detection test again due to the immobilized ChOx does not inhibited by OP compounds. Based on this property, the developed system can be used as a reusable biosensor for OP compound detection.

In this study, bienzyme reaction based OP detection system including ChOx layer and AChE tagged MPs was integrated on a single microdevice, which is composed with microelectrode, microfluidic channel, and external magnet. By using this integrated microfluidic device, the external preparation steps for AChE reaction were eliminated and

*E-mail address: hcyoon@ajou.ac.kr

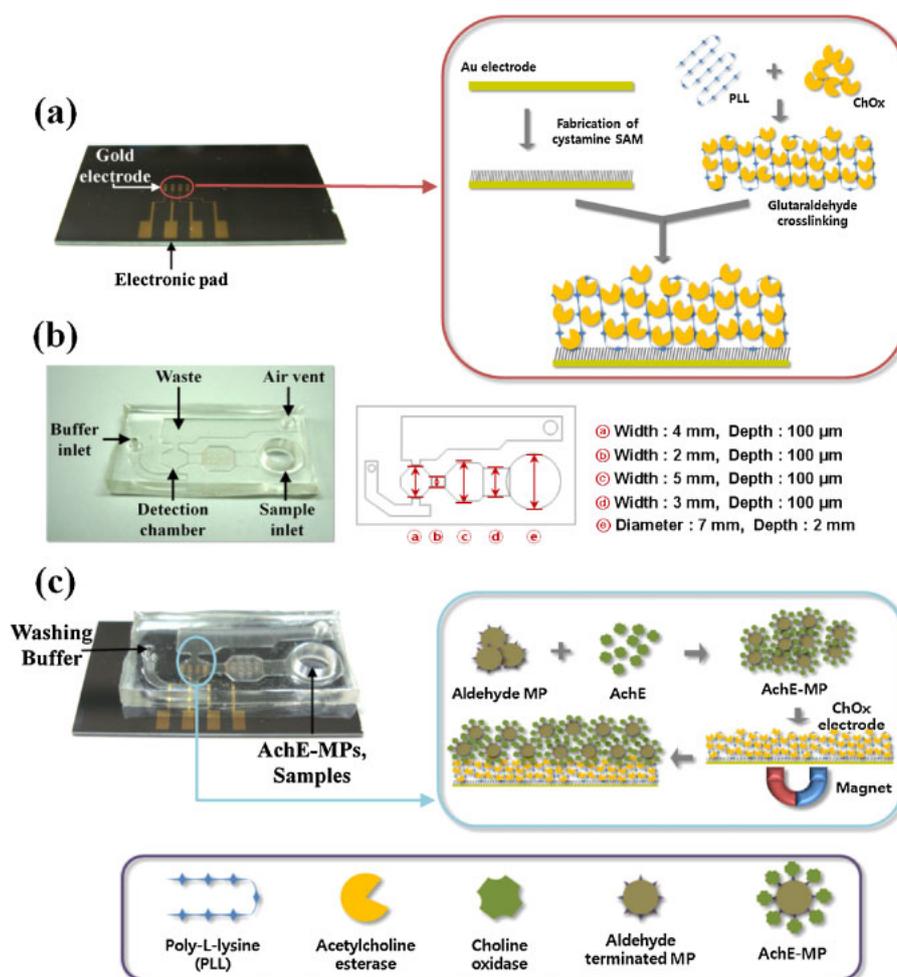


Fig. 2. (Color online) Fabricated microchip for electrochemical OP detection. (a) Micro-patterned Au/Si electrode (left) and ChOx immobilization procedure (right). (b) Picture of PDMS microfluidic channel (left) and its illustration including dimensional description (right). (c) The assembled biosensor chip (left) and AchE-MP arrangement procedure (right).

OP detection steps were simplified. Also, this operational convenience provides a possibility to be used in POCT application for OP detection.

2. Experimental Methods

2.1 Principle of electrochemical OP detection

The electrocatalytic signaling mechanism of the proposed AchE–ChOx bienzyme reaction for OP detection is depicted in Fig. 1. When AchE is inhibited OP, the signal decreases due to the lack of Ch, which acts a substrate for ChOx. In this study, diazinon (DZN), a toxic pesticide, was employed as a model OP compound. The existence of DZN was confirmed and its concentration was measured from the decreased signal. Prior to OP detection, the bioelectrocatalytic mediation of ferrocenemethanol for ChOx and sequential bienzyme reaction of AchE and ChOx were verified.¹⁸⁾ In the absence of enzyme substrate, a typical oxidation and reduction peak of ferrocenemethanol was noted. In the presence of enzyme substrate, the signal was anodically amplified based on the electrocatalytic mediation by ferrocenemethanol. Next, the decrease in bioelectrocatalytic signal was tested using AchE inhibition by DZN. A microfluidic device containing electrochemical working electrodes was designed and fabricated to achieve complicated enzyme-involved reactions including AchE inhibition,

AchE–ChOx cascade reactions, and electrochemical signaling on an integrated system. ChOx was arranged on gold electrodes on the microfabricated sensor component and AchE was arranged on paramagnetic MPs as shown in Fig. 2. AchE-MPs were injected into the the prepared microfluidic chip. A magnet was placed under the ChOx-modified electrode area to localize MPs. ACh was then injected into the microchip and the non-inhibited electrocatalytic signal from the “ACh–normal AchE–ChOx” enzyme cascade reaction was registered. OP (DZN) samples were loaded on to the microchip and reacted with AchE-MPs for five minutes, and the signal from “ACh-inhibited AchE–ChOx” reaction was monitored. Finally, OP concentration was estimated by comparing the signals.

2.2 Fabrication of the microfluidic device

The proposed DZN biosensing platform was prepared on a microfluidic chip as depicted in Fig. 2. It consisted of an Si microfabricated sensor component and a hot-cast poly(dimethylsiloxane) (PDMS) fluidic component. The Si component consists of gold electrodes in an array for immobilization of proteins and electrochemical sensing, all integrated on a 6'' silicon wafer with a 0.1 μm-thick silicon oxide passivation layer [Fig. 2(a)]. The silicon oxide was deposited using low pressure chemical vapor deposition and

tetraethylorthosilicate in a horizontal hot-wall reactor and post-deposition thermal oxidation. Fabrication processes were selected to be simple and suitable for reliable mass production. Six-inch wafer-level silicon complementary metal oxide silicon protocols with three photo-masks were used.^{21,22} In order to fabricate the gold electrodes and pads, thin gold film was patterned with a negative-tone photoresist and then deposited with an electron-beam evaporator of 0.1 μm thickness at 20 °C. The photoresist was then lifted off with acetone for 5 min at 20 °C. The master for replication of PDMS microfluidic component was made of aluminum, and computational numerical control machining protocol was employed to create 100- and 200- μm -deep microchannels in the master.²³ The PDMS solution and a curing agent were mixed (10 : 1 v/v) and poured into the fabricated aluminum master and casted at 70 °C for 2 h in a vacuum oven. Based on the structure of master, the obtained PDMS top substrate included three holes without any punching or cutting procedure [Fig. 2(b)]. The separately prepared Si and PDMS components were then bonded with pressure at 20 °C and hermetically sealed. The freshly prepared PDMS component exhibited satisfactory bonding strength to the silicon oxide surface on the device, and therefore a plasma treatment was unnecessary in this case. The final fabricated microfluidic device chip size was 30 × 20 × 2 mm³ [Fig. 2(c)].

2.3 Fabrication of a ChOx-immobilized electrode

The ChOx-modified electrode was fabricated using the self-assembled monolayer technique and crosslinking (Fig. 2).^{24–27} Poly(L-lysine) (PLL) has a positive charge in neutral solution and forms an electrostatic assembly with the negatively charged ChOx. The addition of glutaraldehyde (GA) as a crosslinker allows Schiff's base to be formed between aldehyde groups from GA and amine groups from PLL and ChOx. Prior to fabricating the ChOx layer on the electrode surface, 100 μL of 5 mM cystamine in double distilled water (DDW) was injected through the inlet hole to form a self-assembled monolayer (SAM). This surface modification was performed for 2 h in a dark container at room temperature. After SAM fabrication, the microchannels and chip surface were washed five times with 200 μL DDW. Nine units of ChOx and 1 mg of PLL were dissolved in 450 μL of phosphate buffered saline solution (PBS; 50 mM phosphate, NaCl 75 mM, pH 7.2). 150 μL of the prepared solution containing PLL and ChOx was mixed with 10 μL of GA (0.2%) as a cross-linking agent. Then, 75 μL of mixture was promptly injected to the inlet hole and reacted with the SAM surface. After a 2 h reaction, the enzyme electrode functionalized with ChOx was rinsed with PBS and stored at 4 °C until use.

2.4 Conjugation of AchE and magnetic particles

AchE was immobilized on the surface of aldehyde-terminated MPs by Schiff's base formation.^{28–30} The aldehyde-terminated MPs were thoroughly washed by resuspending 30 mg of MPs with 1 mL of PBS in a centrifugation tube by vigorous vortexing for one minute. The resulting MP suspension was placed on a magnet to separate the MPs. This MP resuspension step was repeated twice. During the MP washing procedure, AchE stock solution (dissolved in

PBS with 3.8 mg/mL of concentration) was diluted into 1.0, 0.7, 0.4, and 0.1 mg/mL of concentration with PBS. The prepared MPs from the washing procedure were mixed with one mL of AchE solution in various concentrations (0.1, 0.4, 0.7, and 1.0 mg/mL), respectively. Enzyme conjugation was carried out by adding 10 μL of 5 M NaCNBH₃ solution to the MP-AchE suspension and continuously rotating for one hour at room temperature. After the enzyme conjugation procedure, AchE-tagged MPs were washed similarly to the aldehyde-terminated MPs described above, and finally resuspended in 1 mL PBS. The resulting AchE-MPs were stored in the dark at 4 °C until use.

2.5 Selection of AchE-MP deposition site in the microfluidic channel

Various locations in the microfluidic channel were tested as the AchE-MP deposition site in order to maximize the efficiency of the AchE–ChOx enzyme cascade reaction between AchE-MP and ChOx electrodes. Three regions in the microfluidic channel were tested for AchE-MP deposition sites by locating a permanent magnet under these sites [Fig. 3(a)]. An aliquot of 50 μL of AchE-MPs (AchE concentration of 0.1 mg/mL) was loaded into the microfluidic chip through the sample inlet by a micropipette and localized by a magnet. After the MP deposition, PBS was loaded into the buffer inlet hole by a syringe pump to wash the channel. Then, 0.8 mM of ACh solution containing 0.1 mM of ferrocenemethanol was injected through the sample inlet by a micropipette. To minimize the dilution effect by analytes diffusion to the pre-existing buffer solution (PBS) after the washing procedure, a sufficient volume of ACh sample that could drain the washing buffer solution was employed. After the injection of substrate solution, reference electrode and counter electrode were installed in the sample inlet hole for the electrochemical measurement. Then, cyclic voltammetry was conducted with and the electrochemical sensor signal was registered. Voltammograms were recorded at a scan rate of 20 mV/s. All the electrochemical experiments were carried out with an electrochemical analyzer model 630B (CH Instruments) connected to a laptop computer. These conditions for the electrochemical measurement were equally applied to the following studies. The means of the collected signals were calculated, and error bars were used to indicate standard deviations from three independent tests.

2.6 Bioelectrocatalytic signaling and detection of the organophosphorus compound (DZN)

Electrochemical measurements of Ch and ACh in various concentrations were taken in order to explore the electrochemical signaling of the prepared bienzyme electrode. Six different concentrations (0, 0.1, 0.2, 0.4, 0.6, and 0.8 mM) of Ch and ACh solutions were prepared in 0.1 mM ferrocenemethanol-dissolved PBS solution to be used as analytes. Devices were prepared with the aforementioned method in section 2.5 using 0.1 mg/mL of AchE-conjugated MPs. As the MP location region, site #3 was used [Fig. 3(a)]. Once the device was prepared, each of the analyte solutions were loaded into the device through the sample inlet by a micropipette. The loaded volume of AchE-MPs was 50 μL . Cyclic voltammetry was then conducted and the signal was

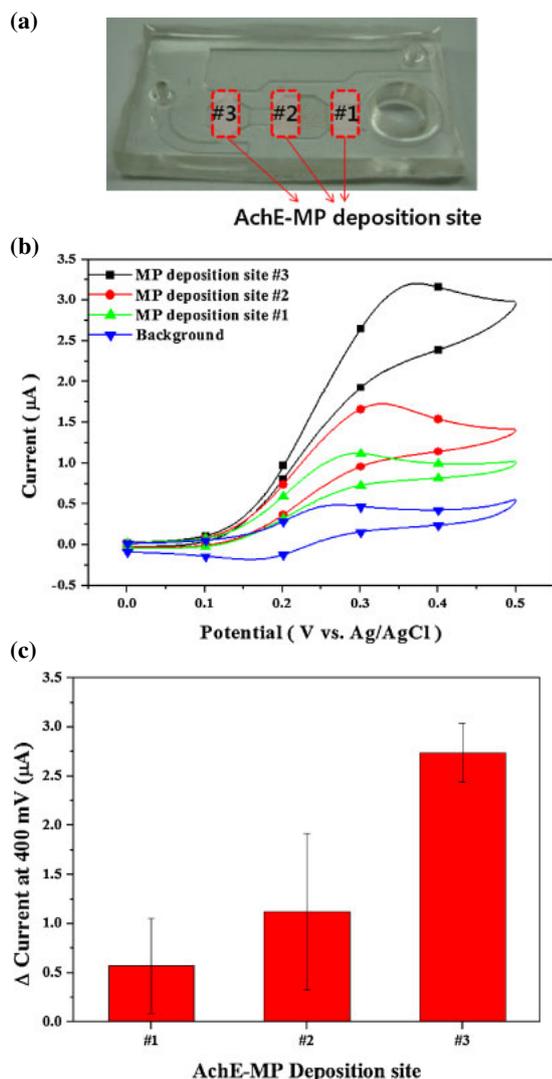


Fig. 3. (Color online) Schematic illustration and comparison of the obtained electrochemical signal for AchE-MP deposition site selection. (a) Locations of AchE-MP deposition sites in the microfluidic channel. (b) Cyclic voltammograms for deposition sites #1, #2, and #3 in the presence of 0.8 mM ACh as a substrate and 0.1 mM ferrocenemethanol as a diffusion mediator; background was the result of deposition site #3 in the absence of ACh. (c) Comparison of registered signal currents at +400 mV vs Ag/AgCl from the background-subtracted voltammograms.

registered. After the electrochemical tests, amplified anodic currents at 400 mV versus the Ag/AgCl reference electrode were sampled from respective voltammograms and collected to obtain calibration curves.

Seven different concentrations (0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 ppm) of DZN solution were prepared for the electrochemical detection of organophosphorus compound. Electrochemical measurement of non-inhibited AchE activity was conducted using 0.8 mM of ACh solution in a prepared microfluidic device containing 50 μL of MP. After measuring the activity of non-inhibited AchE, ACh samples in microchannels were washed by injection of PBS through buffer inlet. Then, DZN samples were loaded into the microfluidic devices through sample inlet using a micropipette and allowed to react for five minutes. After inhibition was completed, microchannels were washed again. Then, a sufficient volume of ACh (0.8 mM) was loaded again into the device to measure the inhibited AchE activity. Independent

triplicate cyclic voltammetric tests were performed. The means of the collected signals were registered, and standard deviations are indicated by error bars.

3. Results and Discussion

Determination of OP concentration is directly related to determination of AchE activity, and therefore a successful bienzyme reaction between AchE and ChOx is important in the AchE inhibition-based OP determination assay. In order to achieve sufficient amounts of Ch product from the AchE reaction, the AchE-MP deposition site in the microfluidic channel should be optimized to account for the fact that AchE and ChOx are immobilized in separate positions in the microfluidic device. Prior to the experiment, we hypothesized the effect of distance between AchE-MPs and ChOx electrode when evaluating AchE activity. If the AchE-MPs were deposited too far away from the ChOx electrode, Ch generated from the AchE-MP reaction would be less likely to reach the ChOx electrode. On the other hand, if MPs were directly deposited on the ChOx electrode surface, the electron transfer between the ferrocene mediator and ChOx could be inhibited by steric hindrance. On the basis of this reasoning, three sites in the microfluidic channel were selected and tested as candidates of the AchE-MP deposition site (Fig. 3). Electrochemical Ch detections were then performed on each microfluidic device using 0.8 mM of ACh as enzyme substrate. Registered voltammograms from the Ch electroanalyses are shown in Fig. 3(b). The signals were collected at +400 mV versus Ag/AgCl from the background-subtracted voltammograms [Fig. 3(c)]. As shown in the results, the bienzyme reaction signal was increased according to the decrease in distance between AchE-MPs and ChOx (from site #1 to #3). The highest signal was observed when the MPs were directly deposited on the ChOx electrode surface (site #3). It appears that the concentration of Ch generated from the AchE enzyme reaction was the same in all three cases because the injected substrate concentration and AchE-MP amount were the same. As Ch from AchE-MPs moves along the channel, Ch molecules are diffused and diluted in the fluids. As the distance between AchE and ChOx increases, the dilution effect of Ch increases and can cause a diffusional limitation of Ch for electrocatalysis. In case #3, an intensively amplified anodic current was developed to deposit the AchE-MP directly on to the ChOx electrode surface. This indicates that the steric hindrance from AchE-MP stacking on the ChOx electrode is not significant, contrary to previous assumption. On the basis of these results, location #3 was employed as the AchE-MP deposition site in all the following experiments.

Sufficient inhibition of loaded AchE is necessary to develop sensitive OP biosensing based on AchE inhibition. If too many AchE molecules are loaded into the OP biosensor, the effect of OP inhibition on AchE would be insignificant. On the other hand, if the amount of AchE is too low, the signal intensity would be low and hard to discern. We modified the concentrations of AchE conjugated to the MPs and measured inhibition rates by applying 0.8 ppm of DZN. The initial and inhibited signals are presented in Fig. 4(a). The % inhibition rate was calculated as shown below, and depicted in Fig. 4(b):

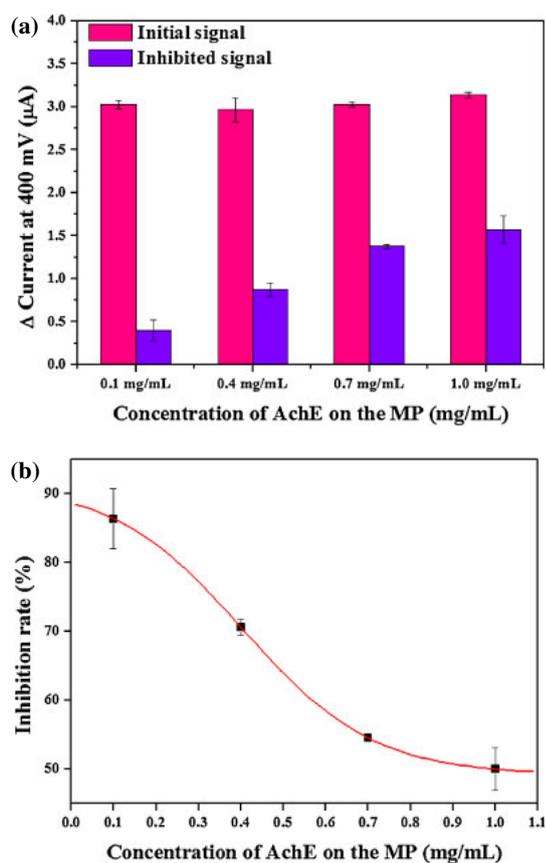


Fig. 4. (Color online) Inhibition of AchE enzyme activity by addition of OP (DZN) at a bienzyme arranged microchip, with four different concentrations of AchE modified MPs. Comparison of normal and inhibited electrochemical signals from the AchE–ChOx bienzyme system with different concentrations of AchE conjugated to MPs. Both signal registrations were achieved by using 0.8 mM ACh as a substrate and 0.1 mM ferrocenemethanol as a diffusion mediator. (a) Comparison of signals from before (initial) and after (inhibited) 0.8 ppm DZN was allowed to react for 5 minutes. Signal registrations were achieved using 0.8 mM ACh and 0.1 mM ferrocenemethanol as a substrate and a mediator respectively. (b) Comparison of % inhibition rates obtained from each of AchE-MPs. Amplified anodic currents at 400 mV vs Ag/AgCl electrode were collected from respective voltammograms and presented in percentile inhibitions.

$$\% \text{ inhibition rate} = \left(\frac{I_n - I}{I_n - I_z} \right) \times 100. \quad (1)$$

Here, I_n is the oxidation current at 400 mV with a non-inhibited enzyme, I_z is the current without the substrate where the enzyme reaction was not induced, and I is the current registered when the enzyme is inhibited by DZN. The inhibited signal increases and the inhibition rate decreases according to the increase in concentration of conjugated AchE [Figs. 4(a) and 4(b)]. When the concentration of AchE that reacts with ACh increases, Ch generation also increases. However, the non-inhibited signals from all test sets were the same, even though the concentration of AchE increased. This suggests that all concentrations of AchE generated sufficient Ch, and that the most effective AchE concentration is that which results in the highest signal change after inhibition by OP (DZN). When compared to the uninhibited initial signal, 0.1 mg/mL of AchE conjugated-MP exhibited the most sensitive inhibition rate (86%) toward 0.8 ppm of DZN. Therefore, AchE at a concentration of 0.1 mg/mL was conjugated to MPs.

Electrochemical signal responses from the fabricated biosensing device to Ch and ACh were evaluated based on the optimized enzyme arrangement. All of the experiments, including AchE-MP deposition, were conducted on the totally assembled microfluidic chip. Six different concentrations of Ch (0, 0.1, 0.2, 0.4, 0.6, and 0.8 mM) samples were prepared as a substrate for the immobilized ChOx during Ch detection. The enzyme reaction of the immobilized ChOx on the electrode in the microfluidic channel with Ch substrate is shown in Fig. 5(a). In the absence of Ch substrate, the electrode exhibited a typical voltammogram for ferrocenemethanol in electrolyte, showing identical oxidation and reduction currents with a 60 mV peak separation. However, in the presence of Ch substrate, the anodically amplified voltammograms correlated with Ch concentration. The signals registered at +400 mV versus Ag/AgCl from the background subtracted voltammograms. Calibrations obtained from cyclic voltammograms of Ch electroanalysis are shown in Fig. 5(b). As shown in the curve, amplified anodic currents were elevated in proportion to the increase in Ch concentration. In the case of Ch detection, the limit of detection and the R^2 value were 10 μ M and 0.98, respectively.

For ACh detection, six different concentrations of ACh (0, 0.1, 0.2, 0.4, 0.6, and 0.8 mM) were prepared as the substrate for the AchE enzyme conjugated to MP. The bienzymatic bioelectrocatalytic reaction between the immobilized ChOx and AchE-MPs within the microfluidic channel with ACh substrate is shown in Fig. 5(c). In this system, there is a linear relationship between the current calibration curve at +400 mV and ACh concentration. Moreover, the anodic current at each concentration of ACh was similar to the current when Ch was used as the substrate, which suggests a highly efficient conversion of ACh to Ch and a successful enzyme cascade reaction. In the case of calibration from ACh detection, the limit of detection and the R^2 value were 7 μ M and 0.99, respectively [Fig. 5(d)]. These observations show that AchE-MPs and ChOx were successfully arranged in the OP biosensing device and that the bienzymatic reaction between AchE and ChOx generated bioelectrocatalytic signals based on the OP interaction with AchE.

A practical OP compound detection using the developed microfluidic biosensing device was created based on the aforementioned conditions and results. Seven different concentrations of DZN (0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 ppm) were used as an analyte for OP detection. The electrocatalytic signal decreased after a five minute reaction of DZN in the microfluidic channel. Figure 6 shows the calibration results from the inhibition of AchE by DZN, as signaled at the prepared device containing the AchE–ChOx bienzyme arrangement. Inhibition rates were calculated using the aforementioned equation [eq. (1)]. Amplified anodic signals were registered at +400 mV versus Ag/AgCl from the background subtracted voltammograms. The limit of detection and the R^2 value of the obtained DZN calibration curve were 8 ppb and 0.97, respectively. Results show a linear detection range that covers concentrations of DZN from 0 ppm to 0.8 ppm, which is required for agricultural and defense fields applications (Fig. 6). Compared to our previous study, this new approach for detection of OP using a bienzyme arranged microfluidic device demonstrates 23% improved sensitivity and 2.5 times

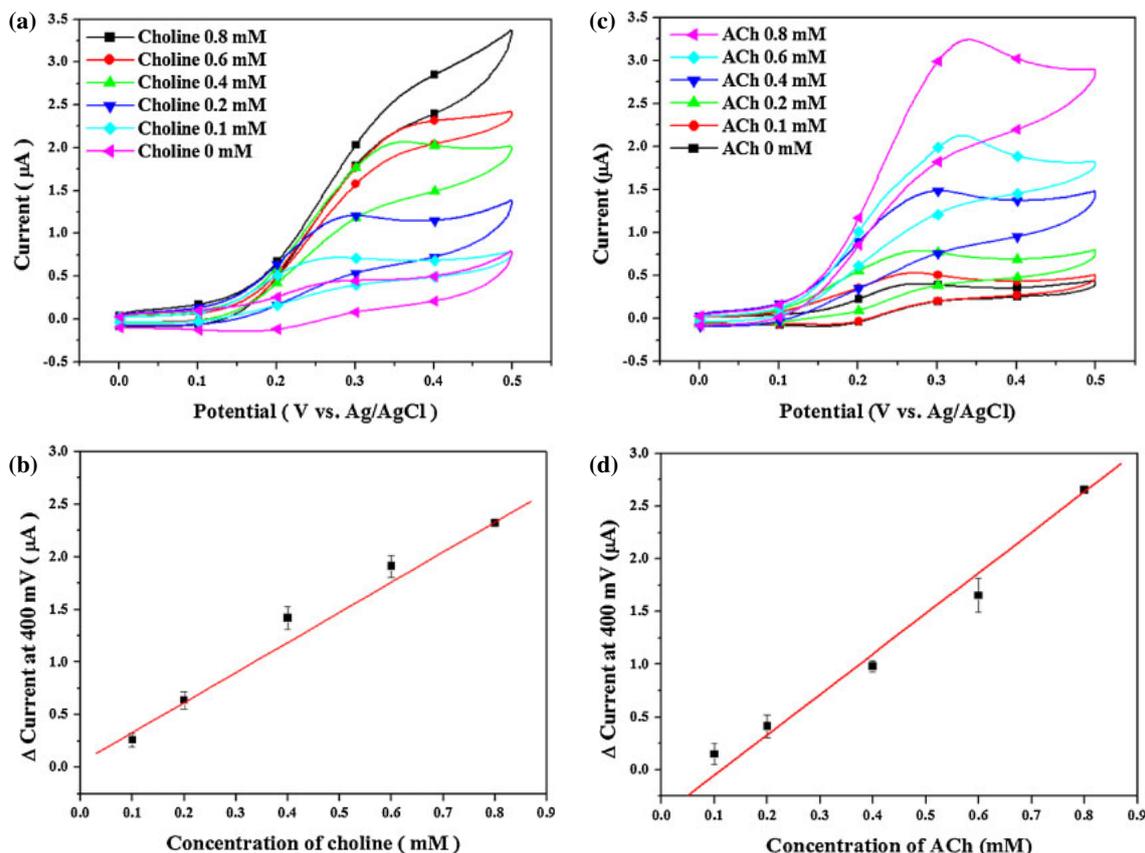


Fig. 5. (Color online) Cyclic voltammetric tests and calibrations from the bioelectrocatalysis of the AChE–ChOx bienzyme system. The system was arranged in the microfluidic device, in the presence of ferrocenemethanol electron-transferring mediator (0.05 mM) in electrolyte. (a) Voltammograms for different Ch concentrations from 0.0 to 0.8 mM are shown. (b) Calibrations for the AChE–ChOx bienzyme biosensing system as a function of Ch concentration. Amplified anodic currents were registered at 400 mV vs Ag/AgCl reference electrode from the background-subtracted voltammograms. (c) Voltammograms for different ACh concentrations from 0.0 to 0.8 mM. (d) Calibrations for the AChE–ChOx bienzyme biosensing system as a function of ACh concentration. Amplified anodic currents were registered at 400 mV vs Ag/AgCl reference electrode from the background-subtracted voltammograms.

enhanced reproducibility.¹⁸⁾ We found the improvements are originated from the concentrated enzyme arrangement and reduced distance between the enzymes, participating in the cascade signaling reactions. Furthermore, due to its simplified and integrated chip structure, all of the OP detection procedures became more simple and convenient. These results suggest that the proposed OP detection system using microfluidic devices and a bienzyme-based bioelectrocatalytic reaction system is useful for convenient and accurate detection of OP compounds.

4. Conclusions

In this study, a novel electrochemical biosensing device for OP compound detection with an AChE–ChOx bienzyme reaction system and integrated microfluidic chip was developed. The PLL-GA crosslinking method was used to immobilize ChOx on the microchip electrode, and a magnetic microparticle was employed for the AChE modification. By combining the covalent bond based enzyme immobilization and magnetism based MP arrangement, the AChE–ChOx bienzyme system was successfully created. In addition, by introducing a microfluidic device, complicated OP detection procedures were accomplished on the single microchip system. Furthermore, through the optimization of AChE arrangement using magnetism, the AChE–ChOx enzyme cascade reaction was induced with

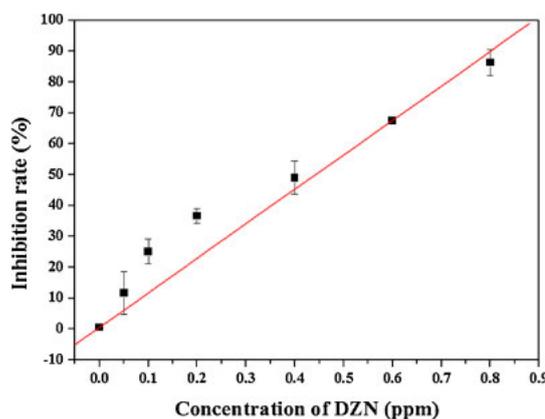


Fig. 6. (Color online) Calibration results from the inhibition of AChE by DZN, ranging from 0 to 0.8 ppm concentrations as registered at the AChE–ChOx bienzyme arranged microfluidic biosensing device. Amplified anodic currents at 400 mV vs Ag/AgCl electrode were collected from respective voltammograms and presented in percentile inhibitions.

high efficiency. These results suggest that the developed bienzyme-modified microchip system can be used as an accurate OP compound detecting biosensor. This integrated microsystem platform can be applied to other biosensing systems that require multi-enzyme reaction.

Acknowledgements

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