Integrated Lateral Flow Test Strip with Electrochemical Sensor for Quantification of Phosphorylated Cholinesterase: Biomarker of Exposure to Organophosphorus Agents

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2012

Impact Factor: 5.64 · DOI: 10.1021/ac202391w · Source: PubMed

5 AUTHORS, INCLUDING:

Dan Du
Washington State University
130 PUBLICATIONS **3,747** CITATIONS
SEE PROFILE

Jun Wang
Nanjing University of Posts and Telecommuni…
355 PUBLICATIONS **11,629** CITATIONS
SEE PROFILE

Donglai lu
Arizona State University
25 PUBLICATIONS **569** CITATIONS
SEE PROFILE

Yuehe Lin
Washington State University
364 PUBLICATIONS **21,422** CITATIONS
SEE PROFILE

analytical chemistry

Article

pubs.acs.org/ac

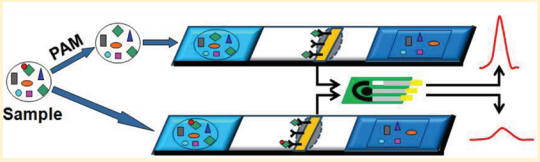
Integrated Lateral Flow Test Strip with Electrochemical Sensor for Quantification of Phosphorylated Cholinesterase: Biomarker of Exposure to Organophosphorus Agents

Dan Du,*[,†,‡] Jun Wang,[‡] Limin Wang,[‡] Donglai Lu,[‡] and Yuehe Lin*[,‡]

[†]Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, PR China

[‡]Pacific Northwest National Laboratory, Richland, Washington 99352, United States

ABSTRACT: An integrated lateral flow test strip with an electrochemical sensor (LFTSES) device with rapid, selective, and sensitive response for quantification of exposure to organophosphorus (OP) pesticides and nerve agents has been developed. The principle of this approach is based on parallel measurements of postexposure and baseline acetylcholinesterase (AChE) enzyme activity, where reactivation of the phosphorylated AChE is exploited to enable measurement of the total amount of AChE (including inhibited and active) which is used as a baseline for calculation of AChE inhibition. Quantitative measurement of phosphorylated adduct (OP-AChE) was realized by subtracting the active AChE from the total amount of AChE. The proposed LFTSES device integrates immunochromatographic test strip technology with electrochemical measurement using a disposable screen printed electrode which is located under the test zone. It shows a linear response between AChE enzyme activity and enzyme concentration from 0.05 to 10 nM, with a detection limit of 0.02 nM. On the basis of this reactivation approach, the LFTSES device has been successfully applied for *in vitro* red blood cells inhibition studies using chlorpyrifos oxon as a model OP agent. This approach not only eliminates the difficulty in screening of low-dose OP exposure because of individual variation of normal AChE values but also avoids the problem in overlapping substrate specificity with cholinesterases and avoids potential interference from other electroactive species in biological samples. It is baseline free and thus provides a rapid, sensitive, selective, and inexpensive tool for in-field and point-of-care assessment of exposures to OP pesticides and nerve agents.



Organophosphorus (OP) compounds have been widely used as pesticides and developed as chemical nerve agents such as soman, sarin, tabun, VX, and others.[1,2] Exposure to even small amounts of OP compounds can be fatal. The mechanism of OP poisoning involves phosphorylation of the serine hydroxyl group in the active site of acetylcholinesterase (AChE) leading to the inactivation of this essential enzyme, which has an important role in neurotransmission.[3,4] At present, detection of OP exposure is generally performed using large, automated analyzers such as liquid or gas chromatography coupled with mass spectrometry (LC/GC/MS).[5,6] Although these methods are sensitive and accurate, they are very expensive, time-consuming, and laboratory-oriented. Therefore, the development of rapid, inexpensive, and field-deployable screening technology for quick response to exposure to OP pesticides and nerve agents is crucial.

Following OP exposure, the agents can readily interact with enzymes and proteins and produce a number of relevant biomarkers including phosphorylated adducts, unbound OP compounds, and hydrolysis products.[7] It is generally recognized that biomonitoring of these typical biomarkers is one of the best efficient approaches for evaluation of OP exposure.[8] Currently, three approaches have been developed including (1) measurement of enzyme activity,[9,10] (2) detection of metabolites by LC/GC/MS,[11−13] and (3) immunoassay of phosphorylated adducts.[7,14−16] Because of the lack of portability and real-time results with LC/GC/MS methods and the scarce availability of OP-specific antibodies in immunoassay, measurement of enzyme activity is the most utilized method for diagnosis of OP exposure.

Enzyme activity assays are relatively simple, as numerous biomonitoring methods have been reported such as the Ellman assay,[17,18] fluorescence assay,[19,20] radiometric assay,[21] Michel (ΔpH) ChE assay,[22] and Walter Reed Army Institute of Research (WRAIR) assay.[23,24] Most recently, an enzyme activity kit (Test-Mate Assay) has been developed and is commercially available for fast screening of OP exposure.[25] All these methods are based on calculating meaningful changes in enzyme activity from a baseline (normal values). A decrease in enzyme activity below 70% of the normal values is an indicator of poisoning with OP agents. However, the normal values vary within laboratories depending on the method of determination.[26] Considering the inter- and intraindividuals variations (e.g., sex, age, ethnicity, etc.) in normal levels of cholinesterase and the deviation methods from different laboratories, these methods do not provide reliable evidence for exposure at

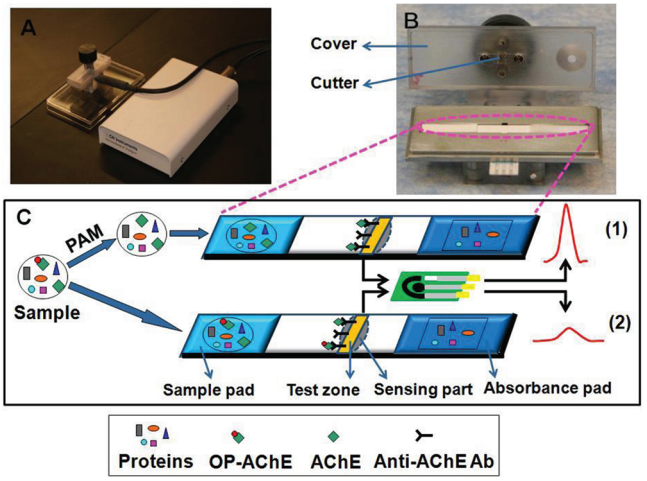
Received: September 9, 2011
Accepted: December 15, 2011
Published: December 15, 2011

   dx.doi.org/10.1021/ac202391w | *Anal. Chem.* 2012, 84, 1380−1385

inhibition levels less than 10−20%.[26] For practical purposes, determination of individual enzyme activity is recommended. Recently, with the reactivation mechanism, a number of assay methods such as measuring released OP from phosphorylated enzyme (fluoride ions-induced reactivation) using MS have been developed for detection and identification of exposure to OPs.[27,28] Oxime-induced reactivation has been used to evaluate drug efficacy and for therapeutic intervention as a treatment for OP poisoning.[29−32] Our group developed a baseline-free method based on reactivation of phosphorylated AChE by oxime to evaluate OP exposure.[33] Treatment of the inhibited AChE with oxime can reverse the inhibited reaction yielding a restored enzyme, which serves as its own control or baseline. Moreover, quantification of exposure to OP agents by simultaneous detection of butyrylcholinesterase (BChE) enzyme activity and total enzyme has been reported.[34] This approach not only eliminates individual variation of BChE values but also avoids the drawback of the scarce availability of OP-specific antibodies.

Recently development of point-of-care (POC) diagnosis biosensors has become a hot research topic. An integrated lateral flow test strip with electrochemical sensor (LFTSES) device offers a new tool for this purpose.[35,36] The LFTSES device has been developed and used as an in-field and POC technology to detect cancer biomarkers, nucleic acids, and biological warfare agents.[37−39] Combining electrochemical detection, LFTSES presents more advantages in miniaturization, low-cost, and high sensitivity. In this paper, we report a new approach for biomonitoring of OP exposure using the LFTSES platform based on parallel measurements of postexposure and baseline AChE enzyme activity (Scheme 1),

Scheme 1. (A) Entire Portable Analytical System, (B) LFTSES Device, and (C) Schematic Illustration of the Principle of the LFTSES Device



where reactivation of the phosphorylated AChE is exploited to enable measurement of the total amount of AChE (including inhibited and active) which serves as a baseline (control). Therefore, the quantification of phosphorylated adduct (OP-AChE) can be realized by subtracting the active AChE from the total amount of AChE. The proposed LFTSES device integrates immunochromatographic test strip technology with electrochemical measurement using a disposable screen printed electrode which is located under the test zone. The signal is further enhanced by using carbon nanotubes (CNTs), which makes the electrochemical detection of the products from enzymatic reactions more feasible at low potentials. This method excludes inter- and intraindividual variation in the normal levels of AChE since a control sample is not needed. It is baseline-free and opens up a new avenue for selective, inexpensive, and rapid POC screening of OP exposures.

EXPERIMENTAL SECTION

Reagents. Human acetylcholinesterase (AChE, 500 U/mg proteins), acetylthiocholine (ATCh), pralidoxime iodide (2-PAM), bovine serum albumin (BSA), phosphate-buffer saline (PBS), and acetone were purchased from Sigma (St. Louis, MO). Chlorpyrifos oxon (CPO) was obtained from Chemical Service, Inc. (West Chester, PA). Anti-AChE antibody (ab17774) was purchased from Abcam Inc. (Cambridge, MA). Human red blood cells (RBCs) were ordered from Biochem. Service (Winchester, VA). A nitrocellulose membrane, absorbent pad, sample pad, conjugation pad, and backing cards were obtained from Millipore (Bedford, MA).

Apparatus. All electrochemical experiments were carried out with a portable electrochemical analyzer CHI 1324 (CH Instruments, Inc., Austin, TX) connected to a laptop. The disposable CNTs modified screen printed carbon electrodes (CNTs/SPCE) were purchased from Dropsens Inc. (Spain). A sensor connector allows for connecting the CNTs/SPCE to the CHI analyzer. The test strip fabrication system consists of an XYZ-3050 dispenser, LM5000 laminator, and the guillotine cutting system CM 4000, which were purchased from BioDot LTD (Irvine, CA). The XYZ-3050 dispenser includes an AirJet Quanti 3000 dispenser and a BioJet Quanti 3000 dispenser.

Test Strip Preparation. The test strip consists of a sample loading pad, nitrocellulose membrane, absorbent pad, and backing card. The sample loading pad was made of glass fiber. Both the sample loading pad and absorbent pad were stored at room temperature without any treatments. The test zone of the strip was prepared by dispensing 1 μL of 1.0 mg/mL anti-AChE antibody onto the nitrocellulose membrane. After drying overnight, the membrane was blocked with 3% BSA for 1 h and then stored at 4 °C. All of the above four parts were assembled on a plastic adhesive backing card using the batch laminating system LM5000. The strips were cut to be 4 mm in width.

Design of LFTSES Device. The laboratory-built LFTSES includes a cover with a cutter, a salver for the test-strip, and a salver for the disposable carbon nanotube modified screen-printed carbon electrode (CNTs/SPCE) which is located under the test zone. The microelectrochemical cell was constructed by sandwiching the CNTs/SPCE in two salvers that were held tightly by magnetic forces using a built-in magnet (Scheme 1B). After immunochromatographic separation and reaction, the captured immunocomplex in the test zone was cut from the test strip by the cutter inside the cover and dropped into the microelectrochemical cell. Followed by adding substrate to the microelectrochemical cell, the electrochemical measurements were conducted to record current responses.

Generation of AChE or RBCs Phosphorylated Adducts and Reactivation. RBCs-AChE was prepared according to Hammond et al.[40] with minor modifications. Briefly, RBCs were lysed in ice-cold water and the cells were then vortexed and incubated for 10 min at 4 °C. The cell membranes were isolated by 8−10 cycles of repeated centrifugation (15 000*g*, 20 min) and rinsing with PBS (10 mM, pH 7.4) until it is only

dx.doi.org/10.1021/ac202391w | *Anal. Chem.* 2012, 84, 1380−1385

slightly pink. The smaller dark red pellet on the bottom is not-lysed RBCs and should be avoided. The CPO stock solution was prepared in acetone and diluted to different concentrations with PBS. Then 40 μL of a series of dilutions of CPO was dispensed into 10 μL of AChE (3 nM) or solubilized RBCs ghost for incubation for 20 min to generate CPO-AChE or CPO-RBCs mixed solution. For reactivation, the above solutions were reacted with 10 μL of 5 mM 2-PAM for 15 min, respectively.

Enzyme Activity Determination on LFTSES Device. A volume of 60 μL of the final reaction solution was cast onto the sample-loading pad and allowed to flow through the whole strip. In the control, AChE (3 nM) or RBC membrane samples were used. After the completion of immunoreactions, the cutter inside the cover was pressed down to cut the test zone with captured AChE to let it insert into the reaction cell. Before electrochemical measurements, 50 μL of 5 mM ATCh was added into the reaction cell. After 2 min of reaction, the square wave voltammetric (SWV) responses were recorded for the quantitative study. The inhibition efficiency (I%) and reactivation efficiency (R%) were calculated with eqs 1 and 2, respectively.

$$I\% = ((i_0 - i_t)/i_0) \times 100\% \quad (1)$$

$$R\% = ((i_r - i_t)/(i_0 - i_t)) \times 100\% \quad (2)$$

where i_0, i_t, and i_r are the peak currents of controlled intact AChE, inhibited AChE, and reactivated AChE. SWV measurements were performed after a 2 s rest period from 0.1 to 0.8 V with a step potential of 4 mV, amplitude of 25 mV, and frequency of 15 Hz.

RESULTS AND DISCUSSION

Reactivation-Based Principle for Quantification of OP Exposure Using LFTSES Device. This approach is based on parallel measurements of postexposure and baseline AChE enzyme activity. As shown in Scheme 1C, one exposure sample (mixture of OP-inhibited and active AChE) is treated with reactivation agent, PAM, and then captured by anti-AChE antibody immobilized on the test strip to give an electrochemical response (line 1). This signal reflects the total amount of AChE activity and serves as a baseline or control (pre-exposure). In parallel, the same sample (mixture of OP-inhibited and active AChE) is directly detected on another test strip to record electrochemical response (line 2), which is only related to the active amount of AChE after exposure to OP. Using the variation value, the phosphorylated adduct (OP-AChE) can be calculated by subtracting the active AChE from the total enzymes. The electrochemical detection of the electroactive enzymatic product thiocholine is based on the following reactions, and the signal is further enhanced by using CNTs.

$$\mathrm{ATCh} + \mathrm{H_2O} \xrightarrow{\mathrm{AChE}} \mathrm{thiocholine(RSH)} + \mathrm{HA(acetic\ acid)} \quad (1)$$

$$2\mathrm{thiocholine(RSH)} \rightarrow \mathrm{thiocholine(RSSR)} + 2\mathrm{H^+} + 2\mathrm{e^-} \quad (2)$$

Reactivation Behavior at LFTSES Device. As shown in Figure 1A, no detectable peak of ATCh was observed on CNTs/SPCE after adding PBS on the test strip (curve a). When 3 nM AChE contained in PBS was dropped onto the test strip for immunoreactions, an obvious oxidation peak on CNTs/SPCE was observed after adding ATCh (curve d). As described before, active AChE was captured by the anti-AChE antibody immobilized on the test zone and cut into the microelectrochemical cell for electrochemical detection using the CNTs/SPCE sensor. This peak results from the oxidation of thiocholine, the hydrolysis product of ATCh which is produced by active AChE. In the following experiment, 50 nM CPO was dispersed to AChE solution (3 nM) in PBS for reaction for 30 min and this mixture liquid was loaded onto the test strip. A greatly decreased current (curve b) was observed compared to active AChE (curve d). It is not strange that exposure to CPO, a model OP, clearly reduced enzyme activity of AChE.

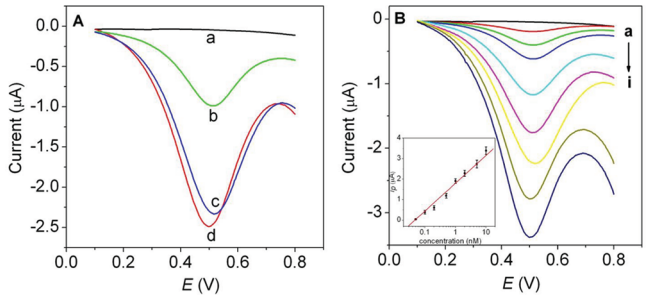


Figure 1. (A) SWV responses on LFTSES device for (a) PBS; (b) mixture of 50 nM CPO and 3 nM AChE in PBS after 30 min of reaction; (c) mixture of 5 mM 2-PAM added to the mixture in part b for 15 min of reaction; (d) 3 nM AChE contained in PBS. (B) SWV responses on LFTSES device with different concentrations of AChE: (a) 0, (b) 0.05, (c) 0.1, (d) 0.2, (e) 0.5, (f) 1.0, (g) 2.0, (h) 5.0, and (i) 10 nM. The inset is the calibration plot for SWV responses and AChE concentrations.

However, after adding 5 mM 2-PAM to the above inhibited AChE solution for 15 min and then dropping it to the test strip, the obtained current (curve c) almost completely regained the level of the active AChE (curve d). As expected for the reactivation procedure, the inhibited AChE has been reactivated completely by the reactivator, 2-PAM. Therefore, this regenerated signal can serve as a baseline or control (pre-exposure) in a given sample. The key for this approach is to establish a calibration curve of AChE enzyme activity vs enzyme concentration (the normalized enzyme activity). Using this calibration curve, the measured postexposure enzyme activity directly correlates with a concentration (molar) of active AChE. For this purpose, a series of standard AChE solutions with different concentrations were loaded onto the LFTSES device for electrochemical measurements. As shown in Figure 1B, the electrochemical signals increased with an increase of AChE concentrations. A linear response was obtained over the concentration range from 0.05 to 10 nM, with the regression equation of i_p (μA) = 1.807 + 1.366 lg c (nM) and the correlation coefficient of 0.9947 (inset of Figure 1B). The detection limit is calculated to be 0.02 nM.

Optimization of Assays. One can see that the inhibition efficiency increased with the increase of inhibition time and reached a plateau after 20 min (Figure 2A). Therefore, 20 min of exposure time for the maximum inhibition was used during experiments. One of the most important parameters is the reactivation time for regeneration of the inhibited enzyme. It was found that the reactivation efficiency increased with increasing reactivation time and tended to a steady value after 20 min (Figure 2B), indicating that the inhibited AChE was

dx.doi.org/10.1021/ac202391w | Anal. Chem. 2012, 84, 1380−1385

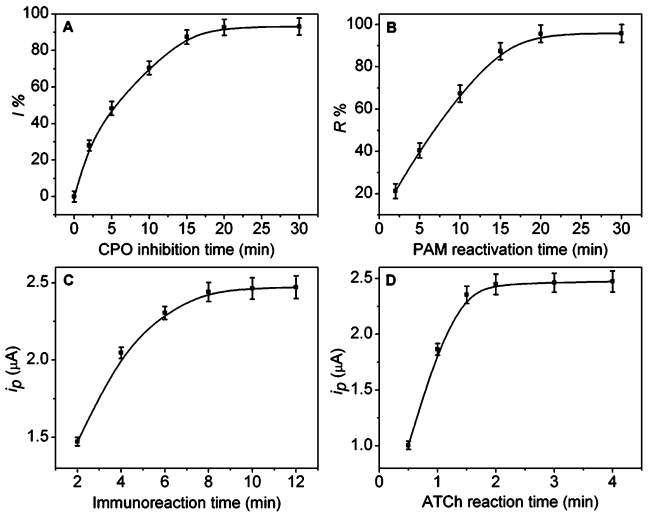


Figure 2. Effect of (A) CPO inhibition time, (B) PAM reactivation time, (C) immunoreaction time, and (D) ATCh reaction time on SWV peak currents. Each sample was measured three times.

regenerated from phosphorylated enzymes and restored its activity. Thus 20 min was used as the reactivation time. The immunoreaction time is another issue for electrochemical measurement. It can be seen that the current responses increase with the immunoreaction time up to 10 min (Figure 2C), indicating a thorough capture of AChE by the anti-AChE antibody immobilized on the test zone. The effect of ATCh reaction time was also studied. As shown in Figure 2D, the electrochemical signal increased greatly upon increasing ATCh reaction time and maintained a very slight increase after 2 min. To balance a high response and less detection time, 2 min was used for the detection performance.

Evaluation of LFTSES Device for Measurement of OP Exposure. To demonstrate the capability of the LFTSES for quantification of phosphorylated adducts based on the reactivation principle, we use standard AChE and postexposure samples for this purpose. Each sample was measured with LFTSES in parallel with and without PAM treatment for detecting enzyme activity, which was then converted to active enzyme concentration using the achieved calibration curve. As shown in Table 1, CPO at varying concentrations (1.0−200 nM) created a broad range of inhibition (5−96%) and decreased AChE activity as well. After treating with 2-PAM, the reactivation efficiency of postexposure samples was higher than 95%. The measured AChE concentrations with regeneration were consistent with the known (control) value. The relative deviation between them is less than 6%, indicating that this new approach is applicable and reliable for evaluation of OP exposure. The phosphorylated adduct was further calculated by subtracting the amount of active AChE from the total AChE. When varying baseline levels of AChE (1 and 5 nM) and varying levels of inhibition, the results are the same as this conclusion.

The device-to-device reproducibility of the proposed method was evaluated by analyzing one sample for three replicate determinations. The coefficients of variation (CVs) were 5.7% and 4.6% at CPO dosages of 5.0 and 50 nM, respectively.

Measurement of OP Exposure in RBCs Samples. The validation of the LFTSES device for measurement of OP exposure was explored with *in vitro* CPO inhibited human RBCs samples. The amount of reactivated AChE in RBCs was measured and the values were consistent with the control. Furthermore, the amount of phosphorylated adduct and the percentage of phosphorylation in these samples could be obtained by this method (Table 2). The reproducibility was further investigated using RBCs samples. The average relative standard deviation (RSD) for the responses was less than 8%, which indicated a reproducible responses.

Age of Phosphorylated Adducts. The "aged enzyme" undergoes dealkylation and is resistant to reactivation. Here we explored the feasibility of regeneration after exposure to CPO at different times (Figure 3). It was found that the inhibited AChE could be restored to ∼90% of the initial activity ∼15 h postexposure, while this reactivation ability decreased very fast after 20 h postexposure (inset of Figure 3). The regeneration efficiency became <30% after 50 h postexposure. As we discussed in our previous paper,[33] the reactivation mode is most suitable for biomonitoring of exposure to slow or nonaging OP pesticides and certain nerve agents such as sarin (half-life is about 10−15 h) and VX (aging is not observed within 24 h). For soman, therapeutic intervention has been difficult due to this rapid aging and stabilization of the phosphorylated adduct. However, the proposed LFTSES device is still promising for the first responders to quickly respond to nerve agent attacks where the diagnosis process for a victim is to be done in minutes rather than hours or days.

■ CONCLUSIONS

We have developed an LFTSES device and demonstrated its low-cost, rapidity, simplicity, and sensitivity for biomonitoring of OP exposure based on reactivation of the phosphorylated AChE. The novelty of this method relies on (1) the use of antibody to selectively capture the enzyme for enzyme activity assay, overcoming the lack of specificity for the conventional

Table 1. Measurements of OP Exposure in Artificially Prepared AChE Samples[a]

sample no.	control	1	2	3	4	5	6
exposure to CPO (nM)	0	1.0	5.0	10	50	100	200
known AChE (c_0, nM)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
I %	0	5.29%	15.2%	21.2%	54.8%	79.8%	96.4%
active AChE after exposure to CPO (c_1, nM)	3.00	2.41 ± 0.1	1.60 ± 0.1	1.25 ± 0.09	0.31 ± 0.04	0.11 ± 0.02	0.05 ± 0.01
total AChE after treating with PAM (c_0', nM)	3.01 ± 0.1	3.02 ± 0.1	2.93 ± 0.2	2.91 ± 0.2	2.90 ± 0.2	2.84 ± 0.2	2.85 ± 0.2
relative deviation $((c_0' - c_0)/c_0) \times 100\%$	0.33%	0.67%	−2.33%	−3.00%	−3.33%	−5.33%	−5.00%
R %		102%	95.9%	96.4%	97.4%	98.3%	98.7%
CPO-AChE adduct ($\Delta c = c_0' - c_1$, nM)		0.61	1.33	1.66	2.59	2.73	2.80

[a]The concentration of the active AChE was calculated by the obtained electrochemical response and the calibration curve. Each sample was measured three times.

dx.doi.org/10.1021/ac202391w | *Anal. Chem.* 2012, 84, 1380−1385

Table 2. Measurement of OP Exposure in Human RBCs Samples[a]

RBCs samples no.	control	1	2	3
dosed CPO (nM)	0	5.0	50	200
active AChE in RBCs after exposure (c_1, nM)	3.19 ± 0.2	2.14 ± 0.1	0.87 ± 0.04	0.36 ± 0.02
total AChE in RBCs after treating with PAM (c_0, nM)	3.12 ± 0.2	3.06 ± 0.2	3.16 ± 0.2	3.00 ± 0.2
CPO-AChE in RBCs ($\Delta c = (c_0 - c_1)$, nM)		0.92	2.29	2.64
AChE phosphorylation ($(\Delta c/c_0) \times 100\%$)		30.1%	72.5%	88.0%

[a]The concentration of the active AChE was calculated by the obtained electrochemical response and the calibration curve. Each sample was measured three times.

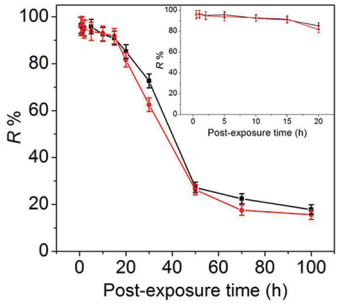


Figure 3. Reactivation efficiency of inhibited AChE vs post-time after exposure to 50 nM CPO. Inset: enlarged plot during 20 h postexposure. Each sample was measured three times.

Ellman assay; (2) the use of test strip for fast immunoreaction and selective separation of AChE from biological samples; (3) the use of reactivating agent for measurement of the total amount of enzyme (including inhibited and active) which served as the baseline, excluding inter- or intraindividual variation in the normal levels of cholinesterase. This approach is baseline free. Overall, the new LFTSES device coupled with a portable electrochemical analyzer shows great promise for in-field and point-of-care diagnosis of OP pesticide poisoning and nerve agent exposure. It is promising for the first responders to use the portable device for rapid identification of the victims in a nerve agent attack for medical treatment.

AUTHOR INFORMATION

Corresponding Author

*E-mail: dudan@mail.ccnu.edu.cn (D.D.); yuehe.lin@pnnl.gov (Y.L.).

ACKNOWLEDGMENTS

The work was done at Pacific Northwest National Laboratory (PNNL) and supported partially by the CounterACT Program, Office of the Director, National Institutes of Health (OD) and the National Institute of Neurological Disorders and Stroke (NINDS), Grant Number U01 NS058161-01. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Dan Du acknowledges the support from the National Natural Science Foundation of China (Grant 21075047). A portion of the research was performed using EMSL, a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory. PNNL is operated by Battelle for the DOE under Contract DE-AC05-76RL01830.

REFERENCES

(1) Black, R. M. *J. Anal. Toxicol.* **2008**, *32*, 2−9.
(2) Giordano, B. C.; Collins, G. E. *Curr. Org. Chem.* **2007**, *11*, 255−256.
(3) Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955−979.
(4) Jarv, J. *Bioorg. Chem.* **1984**, *12*, 259−278.
(5) Gundel, J.; Angerer, J. *J. Chromatogr., B* **2000**, *738*, 47−55.
(6) Hernandez, F.; Sancho, J. V.; Pozo, O. J. *Anal. Bioanal. Chem.* **2005**, *382*, 934−946.
(7) Liu, G. D.; Wang, J.; Barry, R.; Petersen, C.; Timchalk, C.; Gassman, P. L.; Lin, Y. H. *Chem.—Eur. J.* **2008**, *14*, 9951−9959.
(8) Barr, D. B.; Thomas, K.; Curwin, B.; Landsittel, D.; Raymer, J.; Lu, C. S.; Donnelly, K. C.; Acquavella, J. *Environ. Health Perspect.* **2006**, *114*, 936−942.
(9) Mitchell, K. M. *Anal. Chem.* **2004**, *76*, 1098−1106.
(10) Wang, J.; Timchalk, C.; Lin, Y. H. *Environ. Sci. Technol.* **2008**, *42*, 2688−2693.
(11) Noort, D.; Benschop, H. P.; Black, R. M. *Toxicol. Appl. Pharmacol.* **2002**, *184*, 116−126.
(12) Margariti, M. G.; Tsakalof, A. K.; Tsatsakis, A. M. *Ther. Drug Monit.* **2007**, *29*, 150−163.
(13) Wessels, D.; Barr, D. B.; Mendola, P. *Environ. Health Perspect.* **2003**, *111*, 1939−1946.
(14) Wang, H.; Wang, J.; Timchalk, C.; Lin, Y. H. *Anal. Chem.* **2008**, *80*, 8477−8484.
(15) (a) Khattab, A. D.; Ali, I. S. *Environ. Toxicol. Pharmacol.* **2007**, *24*, 275−285. (b) Lu, D.; Wang, J.; Wang, L.; Du, D.; Timchalk, C.; Barry, R. C.; Lin, Y. H. *Adv. Funct. Mater.* **2011**, *21*, 4371−4378.
(16) Sporty, J. L. S.; Lemire, S. W.; Jakubowski, E. M.; Renner, J. A.; Evans, Ronald A.; Williams, R. F.; Schmidt, J. G.; van der Schans, M. J.; Noort, D.; Johnson, R. C. *Anal. Chem.* **2010**, *82*, 6593−6600.
(17) Ellman, G. L.; Courtney, D. K.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88−95.
(18) Kousba, A. A.; Poet, T. S.; Timchalk, C. *Toxicology* **2003**, *188*, 219−232.
(19) Vamvakaki, V.; Fournier, D.; Chaniotakis, N. A. *Biosens. Bioelectron.* **2005**, *21*, 384−388.
(20) Maeda, H.; Matsuno, H.; Ushida, M.; Katayama, K.; Saeki, K.; Itoh, N. *Angew. Chem., Int. Ed.* **2005**, *44*, 2922−2925.
(21) Gordon, R. K.; Doctor, B. P.; Chiang, P. K. *Anal. Biochem.* **1982**, *124*, 333−337.
(22) Ellin, R. I.; Burkhardt, B. H.; Hart, R. D. *Arch. Environ. Health* **1973**, *27*, 48−49.
(23) Gordon, R. K.; Haigh, J. R.; Garcia, G. E.; Feaster, S. R.; Riel, M. A.; Lenz, D. E.; Aisen, P. S.; Doctor, B. P. *Chem. Biol. Interact.* **2005**, *157−158*, 239−246.
(24) Haigh, J. R.; Lefkowitz, L. J.; Capacio, B. R.; Doctor, B. P.; Gordon, R. K. *Chem. Biol. Interact.* **2008**, *175*, 417−420.
(25) Taylor, P. W.; Lukey, B. J.; Clark, C. R.; Lee, R. B.; Roussel, R. R. *Mil. Med.* **2003**, *168*, 314−319.
(26) Jun, D.; Bajgar, J.; Kuca, K.; Kassa, J. Monitoring of blood cholinesterase Activity in workers exposed to nerve agents. In *Handbook of Toxicology of Chemical Warfare Agents*; R. C. Gupta., Ed.; Academic Press: Amsterdam, The Netherlands, 2009; Chapter 58, pp 877−886.
(27) De Jong, L. P.; Van Dijk, C. *Biochem. Pharmacol.* **1984**, *33*, 663−669.
(28) Polhuijs, M.; Langenberg, J. P.; Benschop, H. P. *Toxicol. Appl. Pharmacol.* **1997**, *146*, 156−161.

(29) Bajgar, J.; Kuca, K.; Jun, D.; Bartosova, L.; Fusek, J. *Curr. Drug Metab.* **2007**, *8*, 803−809.
(30) Oha, K. A.; Parka, N. J.; Parka, N. S.; Kuca, K.; Jun, D.; Jung, Y. S. *Chem. Biol. Interact.* **2008**, *175*, 365−367.
(31) Du, D.; Huang, X.; Cai, J.; Zhang, A. D. *Sens. Actuators, B* **2007**, *127*, 531−535.
(32) Du, D.; Huang, X.; Cai, J.; Zhang, A. D. *Biosens. Bioelectron.* **2007**, *23*, 285−289.
(33) Du, D.; Wang, J.; Smith, J. N.; Timchalk, C.; Lin, Y. H. *Anal. Chem.* **2009**, *81*, 9314−9320.
(34) Du, D.; Wang, J.; Wang, L. M.; Lu, D. L.; Smith, J. N.; Timchalk, C.; Lin, Y. H. *Anal. Chem.* **2011**, *83*, 3770−3777.
(35) Guo, Y. C.; Ngom, B.; Le, T.; Jin, X.; Wang, L.; Shi, D.; Wang, X.; Bi, D. *Anal. Chem.* **2010**, *82*, 7550−7555.
(36) Li, Z. H.; Wang, Y.; Wang, J.; Tang, Z. W.; Pounds, J. G.; Lin, Y. H. *Anal. Chem.* **2010**, *82*, 7008−7014.
(37) Liu, G. D.; Lin, Y. Y.; Wang, J.; Wu, H.; Wai, C. M.; Lin, Y. H. *Anal. Chem.* **2007**, *79*, 7644−7653.
(38) Mao, X.; Ma, Y. Q.; Zhang, A. G.; Zhang, L. R.; Zeng, L. W.; Liu, G. D. *Anal. Chem.* **2009**, *81*, 1660−1668.
(39) Zou, Z. X.; Du, D.; Wang, J.; Smith, J. N.; Timchalk, C.; Li, Y. Q.; Lin, Y. H. *Anal. Chem.* **2010**, *82*, 5125−5133.
(40) Hammond, P. I.; Kern, C.; Hong, F.; Kollmeyer, T. M.; Pang, Y. P.; Brimijoin, S. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 190−196.