Full Paper

# Polymeric Membrane Ion-Selective Electrode for Butyrylcholinesterase Based on Controlled Release of Substrate

*Jiawang Ding*,[a,b,c] *Wei Qin*[a,d]*

[a] Laboratory of Environmental Chemistry, Yantai Institute of Coastal Zone Research for Sustainable Development, Chinese Academy of Sciences, Yantai 264003, P. R. China
[b] South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, 510301, P. R. China
[c] Graduate University of the Chinese Academy of Sciences, Beijing 100049, P. R. China
[d] School of Oceanology, Yantai University, Yantai 264005, P. R. China
*e-mail: wqin@yic.ac.cn

Received: January 20, 2009
Accepted: April 24, 2009

**Abstract**
A butyrylcholine selective polymeric membrane electrode using *o*-β-cyclodextrin as ionophore is described as a reagent controlled-release system for reagentless detection of butyrylcholinesterase. Butyrylcholine released across the membrane from inner filling solution of the ion selective electrode is consumed by reaction of the enzyme in sample solution and the concentration of butyrylcholine at the membrane surface can be sensed potentiometrically. The electrode with 0.01 M butyrylcholine in the inner solution yields a potential that varies linearly with butyrylcholinesterase concentration over the range of 0.0075 – 0.15 U $mL^{-1}$ in 0.02 M of pH 7.4 sodium phosphate buffer solution. This approach can also be used to analyze butyrylcholinesterase inhibitors such as organophosphate pesticides. The inhibition percentage of parathion is proportional to its concentration in the range of 0.05 – 0.5 ng $mL^{-1}$ with a detection limit of 0.03 ng $mL^{-1}$.

**Keywords:** Polymer membrane electrodes, Controlled-release, Butyrylcholinesterase, Pesticides, Biosensors, Membranes

DOI: 10.1002/elan.200904635

## 1. Introduction

After pioneering work on polyion-selective electrodes [1] and lower detection limit ion selective electrodes (ISEs) [2], tremendous advances in potentiometric sensors have been achieved during the past decade. It has been fully realized that ions can be transported through polymeric membranes of ISEs when an external voltage or a current is applied, or even under zero-current conditions [3, 4]. Numerous strategies have been developed to fabricate ISE membranes that suffer much less from ion flux effects for large improvement of lower detection limits [5 – 8]. However, it is also clear that, although such ion-flux effects can be strongly reduced, they cannot be entirely eliminated. On the other hand, ion fluxes across the ISE membranes have been found analytically useful, and most of current applications are based on the ion fluxes of primary ions in the direction of inner filling solution. Such examples include polyion sensors [9, 10], pulstrodes [11], switchtrodes [3], ion-channel biosenors [12 – 14], electrodes sensitive to total ion concentrations [15] and ISE indicators for complexometric titrations [16]. These advances have been accompanied by theoretical efforts to understand ion diffusion processes.

In recent years, potentiometric analysis based on outward ion fluxes through ISE membranes, i.e. the fluxes in the direction of sample solution, has been shown to be very promising. Bakker's group reported on the selective coulometric reagent delivery of ions from ionophore based polymeric membranes for calibration-free titrations [17] and investigated the operational limits of controlled current coulometry with these ion-selective membranes [18]. The same group also developed an anion electrode based on precipitate equilibrium at the sample-membrane phase boundary with released silver ions from the sensing membrane [19]. Our group has shown that the outward ion fluxes of ISE membrane can be used as a reagent controlled-release approach for redox sensing chemistries with submicromolar lower detection limits [20]. Such ISE membrane not only serves as a polymer matrix for reagent release, but also works as a transducer for sensitive potentiometric detection. This combination makes the ISE membrane very attractive for sensor miniaturization.

Herein, we present a novel detection system that makes use of outward ion fluxes through ISE membrane to provide a controlled-release substrate for measuring enzyme activity. The constant release of substrate under zero-current conditions from inner solution into sample solution generates a measurable signal in situ. However, the resulting ion fluxes induced membrane potential may be perturbed as a result of enzyme catalysis and thereby the activity of the enzyme can be detected. In this work, butyrylcholinesterase (BuChE) and its substrate butyrylcholine (Buch) were


WILEY InterScience DISCOVER SOMETHING GREAT
© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

chosen as a model system for reagentless biosensing of enzyme activity by ISE. BuChE, which is also named serum cholinesterase, can catalyze the hydrolysis of acetylcholine in the nervous system and thus serves as a coregulator of cholinergic transmission and a new potential target for treating Alzheimer's disease AD [21]. Some compounds such as natural and synthetic drugs and organophosphate pesticides are known to be reversible or irreversible inhibitors of cholinesterases [22–24]. Accordingly, many researchers are hunting for designing of highly sensitive biosensing systems for determination of cholinesterases and their inhibitors [25–29]. Nevertheless, manual addition of substrate to the sample solution is always required before analysis.

In a recent short communication, we have reported that ISE can be used as a controlled reagent delivery system for reagentless biosensing [30]. In this paper, detailed descriptions of the characteristics of this detection system, further monitoring enzyme activity and application toward pesticide determination are presented.

## 2. Experimental

### 2.1. Reagents

Sodium tetrakis[3,5-bis(trifluoromethyl)phenylborate] (NaTFPB), 2-nitrophenyl octyl ether (*o*-NPOE), heptakis(2,3,6-tri-*o*-methyl)-β-cyclodextrin (β-CD) and high molecular weight poly(vinyl chloride) (PVC) were purchased from Fluka AG (Buchs, Switzerland). Butylrylcholine chloride and choline chloride were obtained from Sigma. Butyrylcholinesterase (6.38 U $mg^{-1}$) was purchased from Sangon (Shanghai,China). Parathion (100 μg $mL^{-1}$) was obtained from the National Environmental Monitoring Centre of China. Phosphate buffer solution (PBS) was prepared from 20 mM diabasic sodium phosphate, adjusting with 20 mM monobasic sodium phosphate to reach pH 7.4. All chemicals and reagents were of selectophore or analytical reagent grade. Aqueous solutions were prepared with freshly deionized water (18.2 MΩ specific resistance) obtained with a Pall Cascada laboratory water system.

### 2.2. Membranes and ISEs

The butyrylcholine selective membranes contained 1 wt% NaTFPB, 1 wt% β-CD, 65 wt% *o*-NPOE and 33 wt% PVC, while the ionophore-free membranes contained 1 wt% NaTFPB, 66 wt% *o*-NPOE and 33 wt% PVC. The harder membranes with higher PVC content were prepared with 1 wt% NaTFPB, 1 wt% β-CD, 65 wt% PVC and 33 wt% *o*-NPOE. Membranes of ca. 200 (400) μm thickness were obtained by casting a solution of 180 (360) mg of the membrane components dissolved in 2.0 mL of tetrahydrofuran (THF) into a glass ring of 2.6 mm diameter fixed on a glass plate and letting the solvent evaporate overnight. Membrane thicknesses were visually measured with a CX31-32C02 Olympus microscope (Tokyo, Japan). For each ISE, a disk of 7 mm diameter was punched from the membranes and glued to a plasticized PVC tube (i.d. 6 mm, o.d. 9 mm) with THF/PVC slurry. Measurements for experimental selectivity coefficients and ion fluxes were done by using $10^{-3}$ M NaCl and $10^{-3}$ M Buch as the internal filling solutions, respectively. All the electrodes were conditioned in a solution identical to the inner filling solution overnight. For determination of enzyme and pesticide, $10^{-2}$ M Buch was used as inner filling solution, while 0.02 M pH 7.4 PBS was used sample buffer. After conditioning, the biosensor was repeatedly flushed and soaked in the PBS for 1 hour; when not in use, the sensor was stored in the conditioning solution of $10^{-2}$ M Buch.

### 2.3. Emf Measurements

All measurements of emf were performed at $25 \pm 1\,^{\circ}C$ using a PXSJ-216 pH meter (Shanghai, China) with a saturated calomel electrode as reference electrode in the galvanic cell: SCE//sample solution/ISE membrane/inner filling solution/AgCl/Ag. Selectivity coefficients were determined by the separate solution method in chloride solutions. The emf values for the highest measured ion activities were used for the selectivity coefficient calculation. The activities of butyrylcholine were based on the activity coefficient γ, as calculated from the modified Debye–Hückel equation: $\log \gamma = -0.511\, Z^2\, [\mu^{1/2}/(1+1.5\mu^{1/2}) - 0.2\mu]$, where $\mu$ is the ionic strength and $Z$ is the valence [31].

### 2.4. Measurement of Ion Flux

The released Buch was measured potentiometrically with the butyrylcholine selective electrode. The ion selective electrode with 0.01 M Buch as inner solution was immersed into 5.0 mL of deionized water in a glass vial. With continual stirring of the water solution in the glass vial, the amount of Buch released every two hours in the recipient solution was assayed by the butyrylcholine selective electrode with $1.0 \times 10^{-3}$ M Buch as inner solution.

### 2.5. BuchE Activity and Parathion Determination

The ISE potential was first measured in 0.02 M PBS to obtain a baseline. After addition of BuchE into the buffer solution, the potential change was recorded with time. The potential difference ($\Delta E$) between the baseline and the potential measured at 4 min after enzyme addition was used for quantification of enzyme activity.

The inhibition of parathion was calculated as follows: $I\% = 100\% \times (E_{\text{pesticide}} - E_{\text{enzyme})}/(E_{\text{basline}} - E_{\text{enzyme}})$, where $E_{\text{baseline}}$ is the ISE potential measured in 0.02 M PBS, $E_{\text{pesticide}}$ and $E_{\text{enzyme}}$ are the potentials measured at 8 min after adding 0.03 U/mL BuchE into 0.02 M PBS with and without parathion, respectively.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

## 3. Results and Discussion

### 3.1. Selectivity of β-CD Based Polymeric Membrane Electrode

In our study, the traditional ion selective electrode is used as a controlled-release system for in situ generation and detection of enzyme substrate. Trimethyl *o*-β-cyclodextrin used as ionophore for Buch is suitable for incorporation in the polymeric membrane because of its enhanced lipophilicity, which is caused by the three alkyl groups at the 2, 3 and 6 positions [32]. Based on the toroidal cavity of β-CD, and its molecular recognition for three types of interactions, i.e. direct N–H···O and N–C–H···O hydrogen bondings and van der Waals forces, the better response of the electrode toward Buch can be obtained [33]. The emf response of the polymeric membrane electrode with $10^{-3}$ M Buch as inner solution indicates a Nerstian slope of $57.4 \pm 0.5$ mV per decade in the range of $10^{-1}-10^{-6}$ M Buch. The selectivity coefficients for β-CD based ISE was determined using the method which is termed the "strong interference" method introduced by Bakker [34]. As illustrated in Figure 1, close to theoretical responses are obtained not only for the primary ion but also for the discriminated interfering ions. The emf response of the polymeric membrane electrode toward choline (Cho) and sodium ions are almost the same with and without β-CD, but that toward Buch is enhanced with the ionophore. The logarithmic Nikolskii coefficients for Buch ion ($K_{BuchJ}^{pot}$) over Cho and sodium ions are $-2.54$ ($\pm 0.04$) and $-5.93$ ($\pm 0.05$), respectively. In the case of the membranes without β-CD, the corresponding values are $-1.90$ ($\pm 0.04$) and $-4.90$ ($\pm 0.05$).

### 3.2. Controlled Release of Buch Ions at Membrane Surface

The constant release of primary ions under zero-current conditions from inner solution into sample solution which dictates the detection limit of the potentiometric sensor at low sample concentrations has been extensively studied in recent years [35]. An important issue to be taken account in elucidating the controlled release of ISE is the quantification of the released substrate. In previous research, direct evidence of ionic fluxes across ISE membrane has been tracked by graphite stove atomic absorption spectroscopy [20], fluorescence spectroscopy [36] and scanning electrochemical microscopy [4]. In our system, the ion fluxes of Buch diffusing from the inner solution to the sample solution were potentiometrically monitored by the ISE. It was found that a constant release of Buch could be available after conditioning the electrode overnight. The released rate of Buch was $18 \pm 6$ nmol per hour ($n = 3$).

One more intriguing issue concerning the ion flux is the amount of released butyrylcholine can be modulated. The Buch activities released at the sample-membrane phase boundaries for different membrane electrodes in 0.02 M PBS were measured by calibrating with a series of Buch solutions at higher concentrations. The results are illustrated in Figure 2. It can be seen that higher concentrations of Buch in the inner solution, thinner membranes and higher contents of plasticizers (i.e. softer membranes) could promote the ion fluxes from the inner filling solution into the sample solution and therefore cause higher concentrations of substrate at the sample–membrane interface. Given the ions' diffusion rate in the membrane varies from PVC to poly (n-butyl acrylate) matrix, the ion fluxes of primary ions in the direction of outer solution could also be

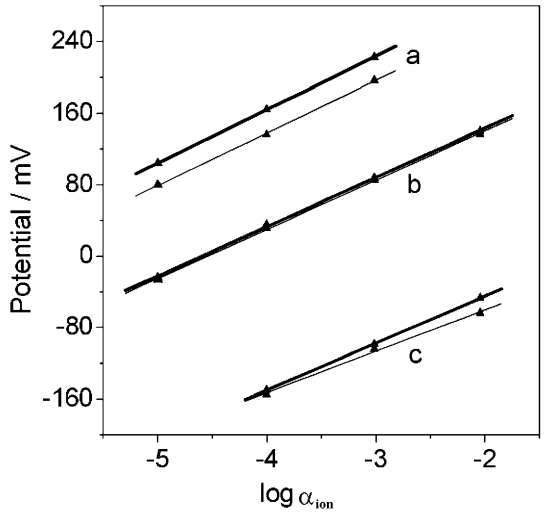


Fig. 1. Emf responses of the ISEs toward a) BuCh, b) Cho, and c) $Na^+$ with (thick line) and without β-CD (thin line) in the polymeric membranes.

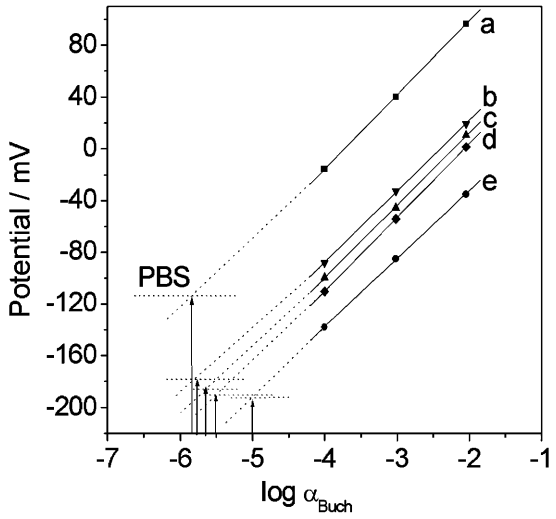


Fig. 2. Emf responses of the ISEs to Buch and 0.02 M PBS with a) $1.0 \times 10^{-4}$ M, b) $1.0 \times 10^{-2}$ M (harder membrane), c) $1.0 \times 10^{-2}$ M (thicker membrane), d) $1.0 \times 10^{-2}$ M, and e) 0.1 M Buch in the inner filling solutions.

 © 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. Effect of PBS concentration on the activity of Buch released at membrane surface of the Buch selective membrane electrode with $1.0 \times 10^{-2}$ M Buch as inner solution.

| PBS concentration (M) | Potential measured [a] (mV) | Activity of Buch released at membrane surface [b] ($10^{-6}$ M) |
|---|---|---|
| 0 | $-246.3 \pm 2.2$ | $0.22 \pm 0.03$ |
| $2.0 \times 10^{-4}$ | $-238.6 \pm 1.9$ | $0.98 \pm 0.03$ |
| $2.0 \times 10^{-3}$ | $-220.3 \pm 1.7$ | $2.14 \pm 0.16$ |
| $2.0 \times 10^{-2}$ | $-195.7 \pm 1.4$ | $3.16 \pm 0.11$ |

[a] Corrected for the liquid potential according to the Henderson equation; mean of three determinations ± standard deviation.
[b] Obtained by calibrating with a series of Buch solutions at higher concentrations of $10^{-4}$, $10^{-3}$ and $10^{-2}$ M; mean of three determinations ± standard deviation.

modulated by changing the matrixes [38]. Moreover, the ion fluxes across the ISE membrane could be controlled by applying an external voltage or a current [3, 17].

### 3.3. Effect of Interfering Ions in Sample Solution

For the present sensor, 0.02 M PBS was added to the sample solution not only to provide the pH buffer suitable for the enzyme catalyzed reaction, but also to maintain the sample ion strength for a constant ion flux across the membrane. Under zero-current conditions, the flux of primary ions diffusing through the membrane is accompanied by that of coextracted counterions in the same direction and/or by a counterflux of interfering ions entering the membrane through an ion-exchange process [39]. The PBS concentration effect on the ion fluxes induced through the ISE membrane is shown in Table 1. It can be seen that the ISE with $10^{-2}$ M Buch as inner solution shows a much lower surface activity of $0.22 \times 10^{-6}$ M in the absence of PBS (i.e. with deionized water), for which the ion flux is mainly caused by the coextraction of Buch and chloride from the inner solution. Increasing the concentration of PBS in the sample solution will increase the concentration of the interfering ion (i.e. $Na^+$), which may partially exchange the primary ions from the sample side of the membrane and induce a concentration gradient through the membrane, thus largely increasing the ion flux. Indeed, the replacement of a very small portion (<1%) of the primary ion can lead to ion fluxes that induce enhanced activities in the stagnant layer near the membrane [40]. This situation is also illustrated in Table 1. In the presence of 0.02 M PBS, the ion flux induced by the ion-exchange process is dramatically accelerated and the activity of Buch at the membrane surface reaches $3.16 \times 10^{-6}$ M. In this case, 1.4% of Buch is replaced by $Na^+$ in the sample side of the membrane as calculated from the selectivity coefficient ($K_{\mathrm{BuchNa}}^{\mathrm{pot}} = 10^{-5.93}$) [41].

### 3.4. Determination of Butyrylcholinesterase Activity

Since butyrylcholinesterase catalyzes the hydrolysis of butyrylcholine, the ISE membrane potential generated by the surface Buch can be sensitive to BuchE. When the ion-selective electrode is immersed into the BuchE solution, the ion flux is disturbed due to the enzyme hydrolysis of butyrylcholine. The potential change, which serves as an indication to the enzyme activity, can be measured by the electrode. For the present work, the remarkable selectivity of Buch selective electrode allowed us to utilize such ISE for monitoring enzyme activity in buffered solution. The maximum potential response ($E_{\max}$) of the electrode is determined by the activity of the Buch ions at the membrane surface, assuming the influence of the background ion activities is negligible:

$$E_{\max} = E^{\circ} + \frac{RT}{F} \ln \alpha_{\mathrm{Buch}} \quad (1)$$

When an enzyme is added into the sample solution, an efficient catalysis reaction occurs and the Buch activity at the phase boundary is decreased, thus decreasing the measured potential. The observed rate of potential change may correlate to the amount of enzyme activity [42]. The minimum potential ($E_{\min}$) will reach if all the Buch ions at the sample-membrane interface are consumed by the enzyme and the sample activities of the interfering cations (i.e. Cho and $Na^+$) govern the electrode response:

$$E_{\min} = E^{\circ} + \frac{RT}{F} \ln \left(\alpha_{\mathrm{Cho}}\, k_{\mathrm{Cho}} + \alpha_{\mathrm{Na^+}}\, k_{\mathrm{Na^+}}\right) \quad (2)$$

As can be seen from Figure 3, with increasing enzyme concentration, the measured potential of the Buch selective membrane electrode is decreased due to the decrease of substrate concentration at the sample-membrane interface. Detailed experimental results reveal that there is a linear dependence of the potential difference ($\Delta E$) after a fixed time on the concentration of enzyme. The linear range is 0.0075 – 0.15 U $mL^{-1}$ ($\Delta E = 1.87 + 66.4 \times C_{\mathrm{enzyme}}$, $r = 0.992$), with a detection limit of 0.006 U $mL^{-1}$ ($3\sigma$). It should be noted that since the ion flux across the ISE membrane is controllable, the sensor's sensitivity and dynamic range to BuchE may be further improved by modulating in situ generation of substrate.

### 3.5. Determination of Organophosphorous Pesticide

The mechanism of cholinesterases inhibition by the pesticides has been widely investigated [43]. It is well known that the organophosphorous pesticide (CX) leads to the phosphorylation of the active site with the concomitant release of the leaving group X (X = *p*-nitrophenol for parathion):

$$\mathrm{E + C - X \rightleftharpoons [EC - X] \rightarrow EC + X} \quad (3)$$

The overall rate constant for the inhibition of the enzyme is given by Equation 4, where [I] is the concentration of the

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

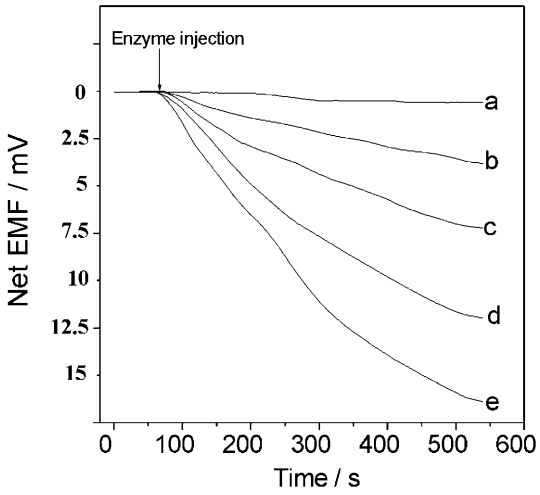


Fig. 3. Potentiometric response of the Buch selective electrode in 0.02 M PBS upon addition of increasing concentrations of BuchE: a) 0, b) 0.0075, c) 0.03, d) 0.075, and e) 0.15 U $mL^{-1}$.

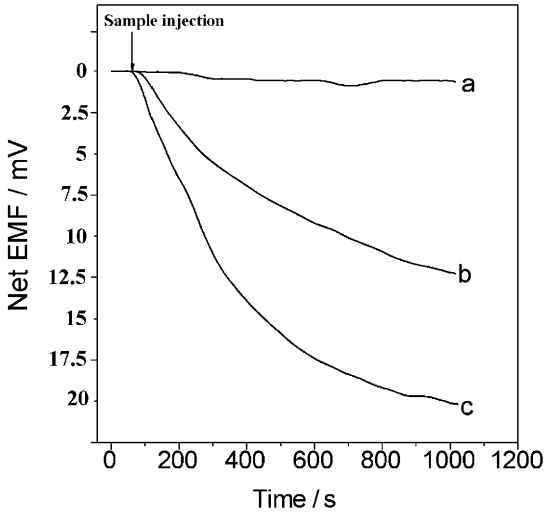


Fig. 4. Potentiometric response of the Buch selective electrode in the blank of 0.02 M PBS a) and in 0.15 U $mL^{-1}$ BuchE sample solutions with b) and without c) 0.01 μg $mL^{-1}$ parathion.

inhibitor, $C_E$ is the concentration of the noninhibited enzyme and $C_{E0}$ is the initial concentration of the enzyme:

$$\ln C_E = \ln C_{E0} - K_i [I] t \quad (4)$$

This equation can be rearranged to give:

$$\ln C_E/C_{E0} = -K_i [I] t \quad (5)$$

To test the application of the sensor, parathion, which has been widely examined as a model of organophosphate pesticide, was determined. As shown in Figure 4, when 0.15 U $mL^{-1}$ BuchE was added into PBS, the potential of the ISE decreased drastically (curve b), as compared with the control (curve a). However, when 0.15 U $mL^{-1}$ BuchE and 0.01 μg $mL^{-1}$ parathion were added into PBS at the same time, the potential change became smaller (curve c). This is due to the fact that parathion as one of the organophosphorous pesticides can be involved in the inhibition action to BuchE thus reducing the enzymatic activity to its substrate. Since the rate of emf change ($dE/dt$) of the response curve in Figure 4 reflects the enzyme activity during the kinetic inhibition of BuchE by parathion in the test solution, Equation 5 can be expressed as:

$$\ln C_E/C_{E0} = \ln (dE/dt)\ (dE_0/dt)^{-1} = K_i [I] t \quad (6)$$

Figure 5 shows the results plotted as a graph of ln ($dE/dt$) ($dE_0/dt$)$^{-1}$ against inhibition time, in which data were obtained from Figure 4. From the slope of the regression line, and knowing the concentration of the inhibitor, the value of $K_i = 3.08 \times 10^5\ M^{-1}\ min^{-1}$ was extracted. This value is in good agreement with the reported value [44]. Under the optimal experimental conditions, the inhibition of parathion on BuchE was proportional to its concentration in the range of 0.05 – 0.5 ng $mL^{-1}$ ($I\ \% = 0.30 + 62.7 \times C_{pesticide}$, $R = 0.995$), with a detection limit of 0.03 ng $mL^{-1}$. The detection limit was one order of magnitude lower than that reported by other researchers [45, 46].

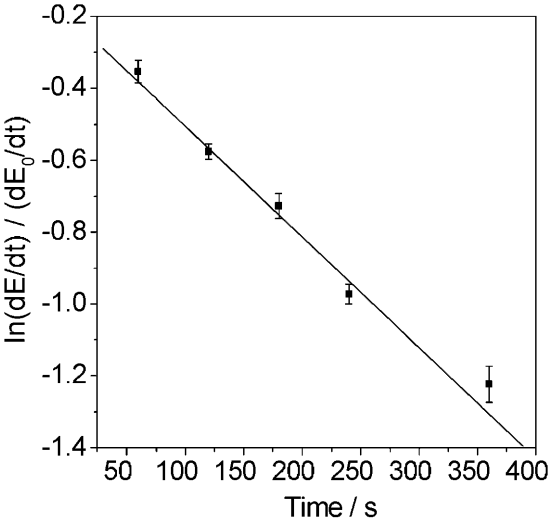


Fig. 5. Results plotted as a graph of ln ($dE/dt$) ($dE_0/dt$)$^{-1}$ (against inhibition time in the presence of 0.01 μg $mL^{-1}$ parathion. Error bars represent one standard deviation for three measurements.

## 4. Conclusions

A butyrylcholine selective polymeric membrane electrode has been employed to develop a simple and real-time cholinesterase assay through in situ generation and detection of enzyme substrate. Applications of this novel approach include the detection of both free and labeled cholinesterase in biosensors and enzyme immunoassays and the measurement of enzyme inhibitors such as organophosphate pesticides. These assays are controllable through modulating ion fluxes across the ion selective membranes.

 © 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Efforts to improve the reproducibility and sensitivity through current-driven measurements are currently in progress in this laboratory.

## 5. Acknowledgements

This work was financially supported by the Chinese Academy of Sciences (KZCX2-YW-410), the National Natural Science Foundation of China (40776058), the National 863 High Technology Project of the Ministry of Science and Technology of China (2007AA09Z103), the Department of Science and Technology of Shandong Province (2006GG2205033), the Outstanding Youth Natural Science Foundation of Shandong Province (JQ200814) and the Taishan Scholar Program of Shandong Province.

## 6. References

[1] S. C. Ma, M. E. Meyerhoff, V. C. Yang, *Anal. Chem.* **1992**, *64*, 694.
[2] T. Sokalski, A. Ceresa, T. Zwickl, E. Pretsch, *J. Am. Chem. Soc.* **1997**, *119*, 11347.
[3] T. Vigassy, W. E. Morf, M. Badertscher, A. Ceresa, N. F. de Rooij, E. Pretsch, *Sens. Actuators B, Chem.* **2001**, *76*, 477.
[4] R. E. Gyurcsányi, É. Pergel, R. Nagy, I. Kapui, B. T. Thu Lan, K. Tóth, I. Bitter, E. Lindner, *Anal. Chem.* **2001**, *73*, 2104.
[5] T. Vigassy, C. G. Huber, R. Wintringer, E. Pretsch, *Anal. Chem.* **2005**, *77*, 3966.
[6] M. Puntener, M. Fibbioli, E. Bakker, E. Pretsch, *Electroanalysis* **2002**, *14*, 1329.
[7] Z. Szigeti, T. Vigassy, E. Bakker, E. Pretsch, *Electroanalysis* **2006**, *18*, 1254.
[8] E. Pretsch, *Trends Anal. Chem.* **2007**, *26*, 46.
[9] M. E. Meyerhoff, B. Fu, E. Bakker, J. H. Yun, V. C. Yang, *Anal. Chem.* **1996**, *68*, 168A.
[10] A. Shvarev, E. Bakker, *J. Am. Chem. Soc.* **2003**, *125*, 11192.
[11] S. Makarychev-Mikhailov, A. Shvarev, E. Bakker, *J. Am. Chem. Soc.* **2004**, *126*, 10548.
[12] P. Reichmuth, H. Sigrist, M. Badertscher, W. E. Morf, N. F. de Rooij, E. Pretsch, *Bioconjug. Chem*. **2002**, *13*, 90.
[13] R. E. Gyurcśanyi, T. Vigassy, E. Pretsch, *Chem. Commun.* **2003**, *20*, 2560.
[14] G. Jágerszki, R. E. Gyurcsányi, L. Höfler, E. Pretsch, *Nano. Lett*. **2007**, *7*, 1609.
[15] A. Ceresa, E. Prestch, E. Bakker, *Anal. Chem.* **2000**, *72*, 2050.
[16] S. Peper, A. Ceresa, E. Bakker,E. Prestch, *Anal. Chem.* **2001**, *73*, 3768.
[17] V. Bhakthavatsalam, A. Shvarev, E. Bakker, *Analyst* **2006**, *131*, 895.
[18] V. Bhakthavatsalam, E. Bakker, *Electroanalysis* **2008**, *20*, 25.
[19] E. Bakker, *Sens. Actuators B, Chem.* **1996**, *20*, 35.
[20] H. M. Guo, T. J. Yin, Q. M. Su, W. Qin, *Talanta* **2008**, *75*, 851.
[21] N. H. Greig, T. Utsuki, D. K. Ingram, Y. Wang, G. Pepeu, C. Scali, Q. S. Yu, J. Mamczarz, H. W. Holloway, T. Giordano, D. M. Chen, K. Furukawa, K. Sambamurti, A. Brossi, D. K. Lahiri, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 17213.
[22] C. G. Zhan, F. Zheng, D. W. Landry, *J. Am. Chem. Soc.* **2003**, *125*, 2462.
[23] D. Suárez, N. Diaz, J. Fontecilla-Camps, M. J. Field, *Biochemistry* **2006**, *45*, 7529.
[24] D. M. Quinn, *Chem. Rev.* **1987**, *87*, 955.
[25] D. Du, J. W. Ding, J. Cai, A. D. Zhang, *Sens. Actuators B, Chem.* **2007**, *127*, 317.
[26] V. B. Kandimalla, H. X. Ju, *Chem. Eur. J.* **2006**, *12*, 1074.
[27] T. Imato, N. Ishibashi, *Biosens. Bioelectron.* **1995**, *10*, 435.
[28] A. L. Ghindilis, T. G. Morzunova, A. V. Barmin, IIya, N. Kurochkin, *Biosens. Bioelectron.* **1996**, *11*, 873.
[29] Y. A. Cho, H. S. Lee, G. S. Cha, Y. T. Lee, *Biosens. Bioelectron.* **1999**, *14*, 435.
[30] J. W. Ding, W. Qin, *Chem. Commun.* **2009**, 971.
[31] S. Kamaata, A. Bhale, Y. Fukunaga, H. Murata, *Anal. Chem.* **1988**, *60*, 2464.
[32] R. Kataky, D. Parker, *Analytst* **1996**, *12*, 1829.
[33] B. N. Barsoum, W. M. Watson, I. M. Mahdi, E. Khalid, *J. Electroanal. Chem.* **2004**, *567*, 277.
[34] E. Bakker, *J. Electrochem. Soc*. **1996**, *143*, L83.
[35] E. Bakker, E. Pretsch, *Trends Anal. Chem.* **2005**, *24*, 199.
[36] W. Qin, W. Zhang, K. P. Xiao, M. E. Meyerhoff, *Anal. Bioanal. Chem*. **2003**, *377*, 929.
[37] G. P. Jin, X. Q. Lin, *Electrochem. Commun.* **2004**, *6*, 454.
[38] A. J. Michalska, Ch. Appaih-Kusi, L. Y. Heng, S. Walkiewicz, E. A. H. Hall, *Anal. Chem.* **2004**, *76*, 2031.
[39] T. Sokalski, T. Zwickl, E. Bakker, E. Pretsch, *Anal. Chem.* **1999**, *71*, 1204.
[40] A. Malon, A. Radu, W. Qin, Y. Qin, A. Cereca, M. Maj-Zurawska, E, Bakker, E. Pretsch, *Anal. Chem.* **2003**, *75*, 3865.
[41] M, Nägele, E, Bakker, E. Ptetsch, *Anal. Chem.* **1999**, *71*, 1041.
[42] H. S. M. Abd-Rabboh, S. A. Nevins, N. Durust, M. E. Meyerhoff, *Biosens. Bioelectron.* **2003**, *18*, 229.
[43] V. Pavlov, Y. Xiao, I. Willner, *Nano Lett.* **2005**, *5*, 649.
[44] Y. C. Chiu, A. R. Main, W. C. Dauterman, *Biochem. Pharmacol.* **1969**, *18*, 2171.
[45] Y. H. Qu, H. Min, Y. Y. Wei, F. Xiao, G. Y. Shi, X. H. Li, L. T. Jin, *Talanta* **2008**, *76*, 851.
[46] Y. H. Liu, M. J. Jin, W. J. Gui, J. L. Cheng, Y. R. Guo, G. N. Zhu, *Anal. Chim. Acta.* **2007**, *591*, 173.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 