



## Biosensor for direct determination of organophosphate nerve agents. 1. Potentiometric enzyme electrode

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### Abstract

A potentiometric enzyme electrode for the direct measurement of organophosphate (OP) nerve agents was developed. The basic element of this enzyme electrode was a pH electrode modified with an immobilized organophosphorus hydrolase (OPH) layer formed by cross-linking OPH with bovine serum albumin (BSA) and glutaraldehyde. OPH catalyses the hydrolysis of organophosphorus pesticides to release protons, the concentration of which is proportional to the amount of hydrolysed substrate. The sensor signal and response time was optimized with respect to the buffer pH, ionic concentration of buffer, temperature, and units of OPH immobilized using paraoxon as substrate. The best sensitivity and response time were obtained using a sensor constructed with 500 IU of OPH and operating in pH 8.5, 1 mM HEPES buffer. Using these conditions, the biosensor was used to measure as low as 2  $\mu$ M of paraoxon, ethyl parathion, methyl parathion and diazinon. The biosensor was completely stable for at least one month when stored in pH 8.5, 1 mM HEPES + 100 mM NaCl buffer at 4°C. © 1999 Elsevier Science S.A. All rights reserved.

**Keywords:** Enzyme electrode; Potentiometric; Organophosphates; Nerve agents; Organophosphorus hydrolase

### 1. Introduction

Organophosphorus (OP) compounds, are among the most toxic substances known (Donarski et al., 1989; Chapalamadugu and Chaudhry, 1992). They are used as pesticides, insecticides and chemical warfare agents (Compton, 1988; FAO, 1989; USDA, 1992). The acute toxicity of organophosphorus neurotoxins and their widespread use in modern agricultural practices has increased public concerns. These concerns have stimulated the development of technologies to treat effluents generated at both the producer and consumer levels effectively (Munnecke, 1980; Coppella et al., 1990; Caldwell and Raushel, 1991; Grice et al., 1996; Mulbury et al., 1996; LeJeune et al., 1997). Additionally, the recently ratified Chemical Weapons Treaty requires the participating nations to destroy all of their chemical weapons arsenal, including the organophosphorus-based nerve gases, within 10 years (Kolakowski et al., 1997;

Rastogi et al., 1997). The successful use of any laboratory developed technology for detoxification of the organophosphate neurotoxins will require analytical tools for monitoring concentrations of these neurotoxins.

Gas, liquid and thin-layer chromatography coupled with different detectors and different types of spectroscopy are the most commonly used methods (Yao et al., 1991; Sherma, 1993). However, these techniques, which are time consuming, expensive and require highly trained personnel, are available only in sophisticated laboratories and are not amenable to on-line process monitoring (Rainina et al., 1996). Biological methods such as immunoassays and inhibition of cholinesterase activity for OP determination have also been reported (Sherma, 1993). Immunoassays (1) require long analysis time (1–2 h) and extensive sample handling (large number of washing steps); (2) require a considerable quantity of costly plastic trays that need to be disposed of in an environmentally acceptable, expensive manner (Pollema and Ruzicka, 1994); and (3) are unsuitable for on-line monitoring.

Biosensing analytical devices, based on acetylcholinesterase (AChE) inhibition, using AChE-modified amperometric (measuring thiocholine and *p*-aminophenol pro-

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duced by hydrolysis of butyrylthiocholine and *p*-aminophenyl acetate, respectively, or hydrogen peroxide generated as a result of the oxidation of choline produced from acetylcholine hydrolysis in the presence of choline oxidase) (Skladal, 1991; Marty et al., 1992; Palleschi et al., 1992; Skladal and Mascini, 1992; La Rosa et al., 1994; Martorell et al., 1994; Mionetto et al., 1994; Trojanowicz and Hitchman, 1996; Martorell et al., 1997; Palchetti et al., 1997), potentiometric (measuring the pH change as a result of acetic acid production) (Tran-Minh et al., 1990; Chuna Bastos et al., 1991; Kumaran and Tran-Minh, 1992; Kumaran and Morita, 1995; Danzer and Schwedt, 1996), conductometric (Dzyadevich et al., 1994) or fiber optic (monitoring the pH change using pH indicators or the formation of chromophoric product) (Rogers et al., 1991; Hobel and Polster, 1992; Trettnak et al., 1993; Garcia de Maria et al., 1994; Moris et al., 1995; Andres and Narayanaswamy, 1997) transducers have been reported. Although sensitive and useful as single use disposable sensors for environmental monitoring, biosensors based on AChE inhibition have the following limitations for on-line process monitoring application: (1) these biosensors have a long and tedious protocol that requires long incubation with inhibitors prior to analysis for good sensitivity, and treatment with pyridine-2-aldoxime after analysis partly to regenerate/recover the enzyme activity which is inhibited irreversibly by OP (Marty et al., 1992; Dzyadevich et al., 1994; La Rosa et al., 1994); and (2) since AChE is inhibited by neurotoxins, which include not only OP pesticides but also carbamate pesticides and many other compounds, these analytical tools are not selective and cannot be used for quantitation of either an individual or a class of pesticides that may be required for monitoring detoxification processes.

Organophosphorus hydrolase (OPH), a biological catalyst, has been shown to effectively hydrolyse a range of organophosphate esters, pesticides such as parathion, coumaphos and acephate, and chemical warfare agents such as soman, sarin, VX and tabun (Lewis et al., 1988; Donarski et al., 1989; Dumas et al., 1989a, b, 1990; Dave et al., 1993; Lai et al., 1994). The catalytic hydrolysis of each molecule of these compounds releases two protons, the measurement and correlation of which to the OP concentration, forms the basis of a potentiometric enzyme electrode. The detection scheme based on monitoring the OPH-catalysed hydrolysis products of OPs offers the advantages of simpler, more direct, and quicker measurements of only the organophosphorus class of nerve agents over that based on the inhibition of acetylcholinesterase activity that responds to all types of nerve agents. Additionally, the OPH-based biosensor has the potential of quantifying individual organophosphate pesticides when used as a detector in conjunction with high-pressure liquid chromatography (HPLC) for chromatographic separation.

Recently, a couple of potentiometric biosensors for the direct determination of organophosphates were reported (Rainina et al., 1996). These sensors consisted of OPH-expressing recombinant *Escherichia coli* cells cryoimmobilized by entrapment in poly(vinyl)alcohol gel. The immobilized cells were either suspended in a batch reactor with a pH electrode or packed in a column and placed upstream of a flow-cell with a pH electrode. The long response time that is attributable to the various mass transfer resistances present in the system and the need of a special equipment for cryoimmobilization of the cells are the limitations of the reported biosensing systems.

This paper describes the construction of a simple potentiometric enzyme electrode for the direct, sensitive, selective and rapid determination of organophosphate nerve agents, using OPH immobilized via crosslinking on the surface of a pH electrode that can potentially be used for on-line monitoring of detoxification processes.

## 2. Materials and methods

### 2.1. Reagents

HEPES, yeast extract, tryptone, ammonium sulfate, potassium monobasic phosphate, potassium dibasic phosphate, cobalt chloride, glutaraldehyde, isopropanol, and glycerol were purchased from Fisher Scientific (Tustin, CA, USA). Bovine serum albumin (BSA), polyethyleneimine, lysozyme and phenyl-methylsulphonyl fluoride (PMSF) was obtained from Sigma Chemical Company (St Louis, MO, USA). Paraoxon, parathion, methyl parathion, diazinon, sevin, sutan, atrazine, and simazine were acquired from Supelco Inc. (Bellefonte, PA, USA). Dialysis membrane was purchased from Spectrum Medical industries, Inc. (Los Angeles, CA, USA). Chromatography packing material, Sephadex G-150 and DEAE-Sephadex A-50, were obtained from Pharmacia Biotech (Uppsala, Sweden). All the solutions were made in distilled deionized water.

### 2.2. OPH production and purification

OPH was purified from recombinant *E. coli* carrying plasmid pJK33 (obtained from Dr Jeffrey Karns, USDA, Beltsville, MD, USA) according to the reported protocol (Omburo et al., 1992). In brief, cells from 3 l of culture broth, grown to stationary phase (35–38 h) at 30°C in medium containing 12 g l<sup>-1</sup> tryptone, 24 g l<sup>-1</sup> yeast extract, 0.4% (v/v) glycerol, 80 mM K<sub>2</sub>HPO<sub>4</sub> and 20 mM KH<sub>2</sub>PO<sub>4</sub>, were harvested by centrifugation at 8000g for 10 min at 4°C, washed with distilled water and resuspended in two pellet volumes of buffer A (pH 8.5, 50 mM HEPES buffer + 50 μM CoCl<sub>2</sub>) containing 10 μM of protease inhibitor PMSF, dissolved in isopropanol).

After adding 10  $\mu\text{g}/\text{ml}$  of lysozyme, the cells were lysed using 15 s pulsed sonication for 30 min at 0°C and medium power setting using a model 350 Sonifier Cell Disruptor (Branson Sonic Power, Danbury, CT, USA). The lysed cell suspension was then centrifuged at 13 000g for 1 h at 4°C (the temperature of all the subsequent steps) to recover the enzyme containing supernatant. A 2% polyethyleneimine (PEI) solution was then added dropwise with stirring to the supernatant over a period of 30 min until the PEI concentration was 0.4%. The solution was stirred for an additional 30 min, and the mixture was centrifuged at 13 000g for 15 min. The supernatant solution was then subjected to ammonium sulfate fractionation by the addition of 258 mg of solid ammonium sulfate per ml of protein solution (45% of saturation) over a period of 30 min with stirring. After an additional 30 min stirring, the mixture was centrifuged at 13 000g force for 20 min to recover the pellet containing the OPH. The pellet was then dissolved in 10 ml of buffer and loaded onto a 2.5  $\times$  90 cm column containing Sephadex G-150 equilibrated with buffer A. Five milliliter fractions of the column eluant, eluted at a flow rate of 1 ml  $\text{min}^{-1}$ , were collected and pooled on the basis of enzymatic activity and absorbance at 280 nm (due to protein). The pooled fractions were subsequently loaded onto a 2.5  $\times$  30 cm column containing DEAE-Sephadex A-50 equilibrated with buffer A. Two-and-a-half milliliter fractions of the column eluant, eluted at a flow rate of 1 ml  $\text{min}^{-1}$ , were collected and fractions pooled on the basis of enzyme activity and protein concentration. The pooled fractions were concentrated (1–2 ml) by lyophilization, dialysed against buffer A and concentrated again using a speed vac. The purified enzyme (7250 IU  $\text{mg}^{-1}$  protein and 15 mg  $\text{ml}^{-1}$  protein) was then stored at 4°C. The progress of purification was monitored by determining the specific activity ( $\mu\text{mol}$  of *p*-nitrophenol (pNP) formed per min per mg protein, during hydrolysis of 1 mM paraoxon in pH 8.5 buffer at 30°C; the rate of pNP formation was followed by measuring the rate of increase of the absorbance at 400 nm ( $\epsilon_{400} = 17\,000\, \text{M}^{-1}\, \text{cm}^{-1}$ ).

### 2.3. OPH-based electrode construction

An OPH-based potentiometric enzyme electrode was constructed by immobilizing OPH directly on the hydrogen ion sensing glass membrane of the pH electrode (Accumet, model 13-620-289, Fisher Scientific, Tustin, CA, USA). An aliquot of 10  $\mu\text{l}$  from a 14  $\mu\text{l}$  mixture prepared by mixing 2  $\mu\text{l}$  of 10% BSA, 2  $\mu\text{l}$  of 2.5% glutaraldehyde (a bifunctional protein cross-linker) and 10  $\mu\text{l}$  of buffer containing different amounts of OPH was spread on the surface of the pH electrode held upside-down in a clamp and allowed to dry for 30–40 min; a yellow color cross-linked (due to Schiff-base reaction between the  $\text{NH}_2$  of lysine and CHO of

glutaraldehyde) protein gel was formed. Subsequently, the gel was covered with a 12–14 kDa molecular weight cut-off dialysis membrane, which was held in place by an O-ring, and washed thoroughly with buffer (pH 8.5, 1 mM HEPES plus 100 mM NaCl and 50  $\mu\text{M}$   $\text{CoCl}_2$ ) to remove any excess glutaraldehyde.

### 2.4. Experimental setup and measurement

The experimental setup is schematically depicted in Fig. 1. All measurements were made in 5 ml of an appropriate buffer, thermostated to the desired temperature, in a 10 ml working volume jacketed glass cell, equipped with a magnetic stirrer. The temperature of the liquid in the cell was controlled by circulating water in the cell jacket using a circulating water bath (model 1160, VWR Scientific, San Francisco, CA, USA). Five to 10  $\mu\text{l}$  of OP nerve agent, dissolved in pure methanol, was added to the cell and the changes in potential, i.e. pH, were recorded with a pH/ion analyser (model 255, Corning Science Products, Corning, NY, USA) connected to a flat bed chart recorder (model BD112, Kipp and Zonen, Holland).

## 3. Results and discussion

### 3.1. Effect of various parameters on the response of enzyme electrode

The sensitivity, change of potential per unit change in analyte concentration, and response time of a potentiometric enzyme-electrode are functions of the buffer concentration, starting pH of buffer, temperature and the units of enzyme immobilized on the surface of the electrode. Experiments were performed to investigate the effect of these variables on the rate of change of the initial response (determined by drawing a tangent to the pH vs. time response curve) of the electrode to 100  $\mu\text{M}$  injections of paraoxon.

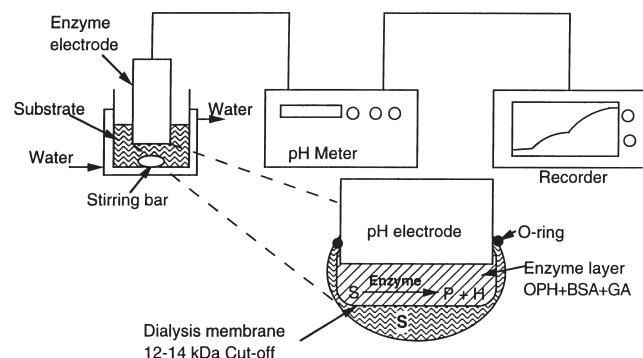


Fig. 1. Schematic drawing of the potentiometric enzyme electrode.

### 3.2. Effect of buffer concentration

The buffer concentration has a marked influence on the rate of potential change, which was an inverse function of the buffer concentration (Fig. 2). The inverse relationship is due to the fact that a higher concentration buffer counteracts the pH change resulting from protons released during the OPH-catalysed hydrolysis of organophosphate nerve agents better than a lower concentration buffer. Although the magnitude of the response, the lower detection limit and the response time of the electrode were better in the weak buffer, the linear dynamic concentration range was better in a stronger buffer (0.3–1 mM in 5 mM buffer compared with 0.15–0.7 mM in 1 mM buffer). These results are in agreement with those observed for other potentiometric biosensors (Brand et al., 1989) and OPH-based microbial biosensor for OPs (Rainina et al., 1996). Since an objective of this work was to develop a rapid and sensitive biosensor for organophosphate nerve agents, 1 mM buffer was selected for subsequent investigations. Because the 1 mM buffer was unstable and rather difficult to work with, 100 mM sodium chloride was added to it to make it stable. The addition of this neutral salt made the buffer easier to work with without affecting the enzyme electrode response characteristic (data not shown).

### 3.3. Effect of starting pH

The pH profiles for the OPH-modified enzyme electrode and the free enzyme are similar (Fig. 3). This fact, in conjunction with the observation that there was no pH

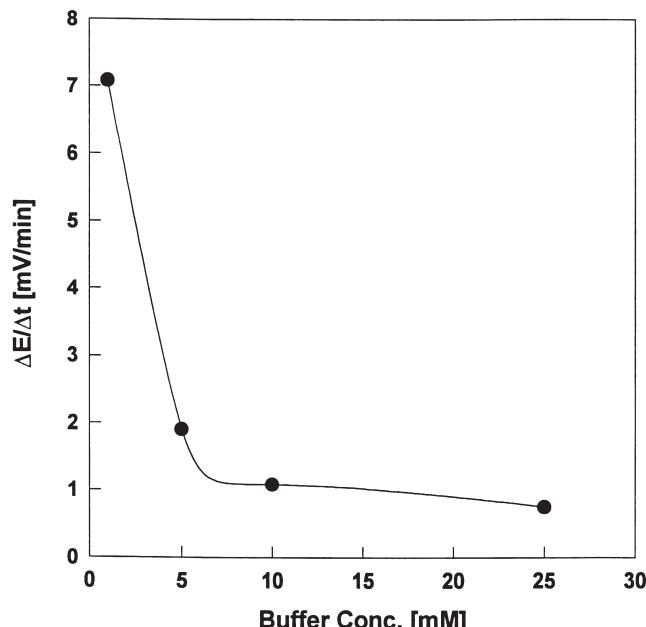


Fig. 2. Effect of buffer concentration on the response of the enzyme electrode to 0.1 mM paraoxon in pH 8.5 HEPES buffer with 0.05 mM  $\text{CoCl}_2$  at 20°C. Enzyme amount: 775 IU.

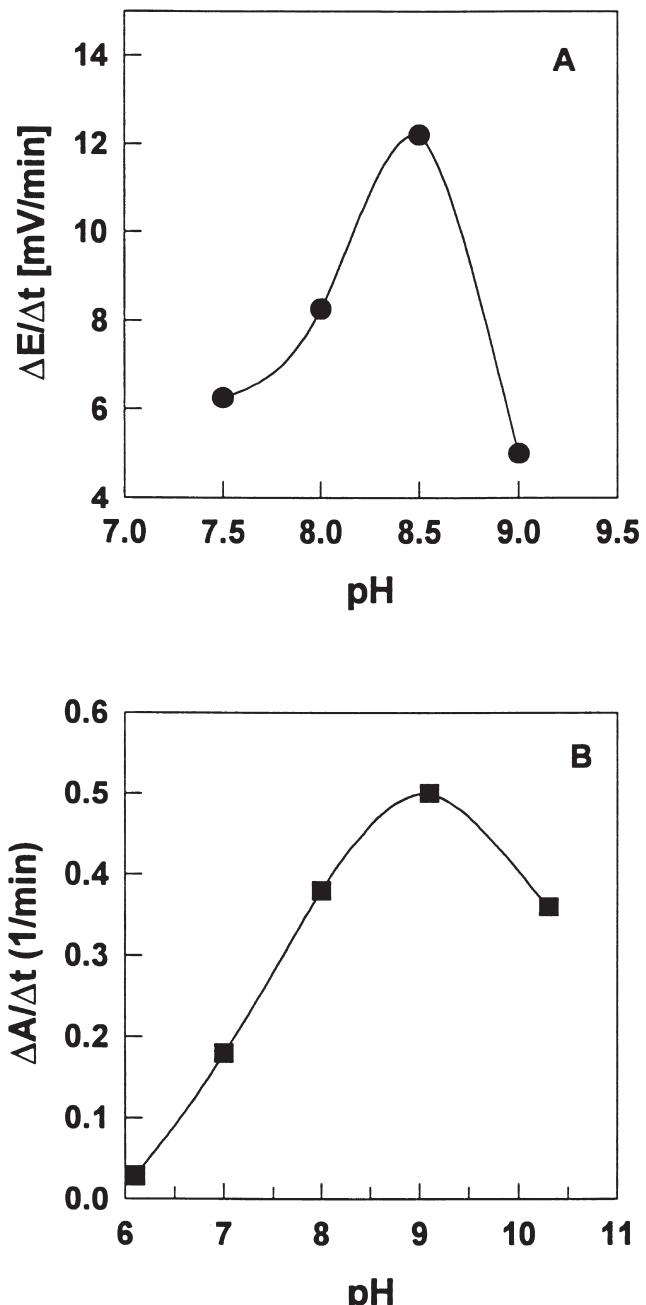


Fig. 3. (A) Effect of buffer starting pH on the response of the enzyme electrode to 0.1 mM paraoxon in 1 mM HEPES + 100 mM  $\text{NaCl}$  + 0.05 mM  $\text{CoCl}_2$  buffer at 20°C. Enzyme amount: 775 IU. (B) Effect of pH on the activity of free OPH determined in 50 mM HEPES + 0.05 mM  $\text{CoCl}_2$  at 30°C with 0.07 mM paraoxon.

change in the control experiment (when the enzyme layer was absent), demonstrates that the observed pH dependence of the sensor response is due to the pH dependence of the OPH activity. The 0.5 unit difference between the optimum for the free and immobilized enzyme can be attributed to an alteration of the physico-chemical characteristics of the enzyme due to immobilization (Mulchandani et al., 1990). pH 8.5, that gave the

maximum sensitivity, lowest response time and largest dynamic range was used subsequently.

### 3.4. Effect of temperature

Fig. 4 shows the effect of temperature on the response of the OPH-modified potentiometric enzyme electrode. As depicted, response increased with temperature up to 45°C and then decreased with further temperature increase. The initial increase in the rate is attributed to the increase of both the enzyme reaction and mass transport rates. The decrease in the rate at higher temperatures is due to enzyme denaturation. This temperature profile is in accordance with that reported for the free enzyme (Rowland et al., 1991). Although 45°C was determined to be the optimum temperature for the enzyme electrode operation, subsequent experiments were still performed at room temperature, 20°C. This was done in order to overcome the problem of excessive evaporative losses during the course of the experiment and ease of operations.

### 3.5. Effect of enzyme loading

While maintaining a constant total protein concentration of 2%, using BSA, so as to eliminate variations in the properties of the resulting enzyme film, the OPH loading on the electrode was varied and the rate of potential change measured. As can be seen in Fig. 5, increasing the enzyme loading above 388 units did not result in any further improvement in the rate of potential change. This profile is in accordance with the model for

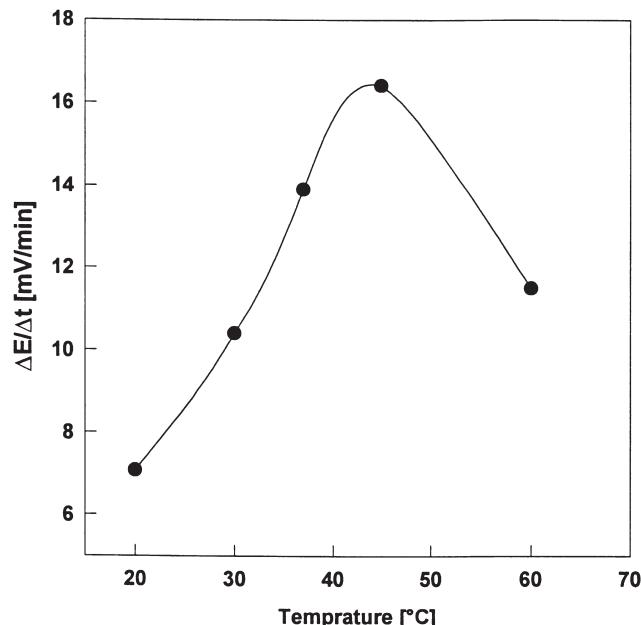


Fig. 4. Effect of temperature on the response of the enzyme electrode to 0.1 mM paraoxon in pH 8.5, 1 mM HEPES + 100 mM NaCl + 0.05 mM CoCl<sub>2</sub>. Enzyme loading: 775 IU.

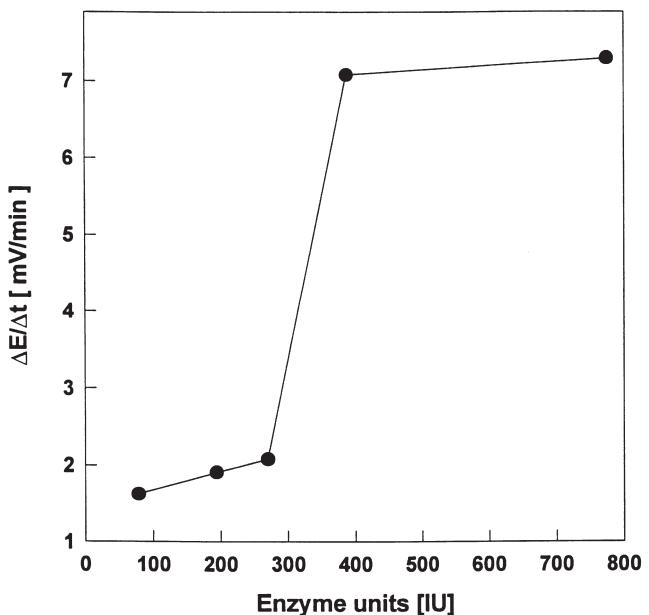


Fig. 5. Effect of enzyme loading on the response of the enzyme electrode to 0.1 mM paraoxon in pH 8.5, 1 mM HEPES + 100 mM NaCl + 0.05 mM CoCl<sub>2</sub> at 20°C.

enzyme electrode, in which the product of enzyme-catalysed reaction is detected by a non-reactive detector, i.e. potentiometric (Eddowes, 1990). Below 388 units of the enzyme on the electrode surface, the enzyme reaction rate is the rate-controlling step, and increasing the amount of the enzyme results in increased sensitivity and shorter response time. However, above 388 units of enzyme mass transport becomes the rate-controlling step and no further increase in sensitivity or decrease in response time can be obtained. The mass transfer controlling region is preferred for an enzyme electrode since in this region the response of the electrode becomes relatively insensitive to changes in enzyme activity and thus prolongs the biosensor operational and storage lifetime (Carr and Bowers, 1980). An OPH loading of 500 units was therefore selected for all subsequent experiments.

## 3.6. Analytical characteristics of enzyme electrode

### 3.6.1. Calibration plots for organophosphates

Fig. 6 shows the calibration plots for paraoxon, parathion, methyl parathion and diazinon using the potentiometric enzyme electrode (these plots were prepared from the steady-state response data). The important characteristics for the different analytes measured using the OPH-based potentiometric enzyme electrode are summarized in Table 1. The maximum sensitivity of the enzyme electrode was in the order paraoxon > methyl parathion  $\approx$  parathion > diazinon. The trend is in agreement with model prediction (Eddowes, 1990) based on the reported  $V_{max}/K_M$  values for these compounds (Mason et al., 1997).

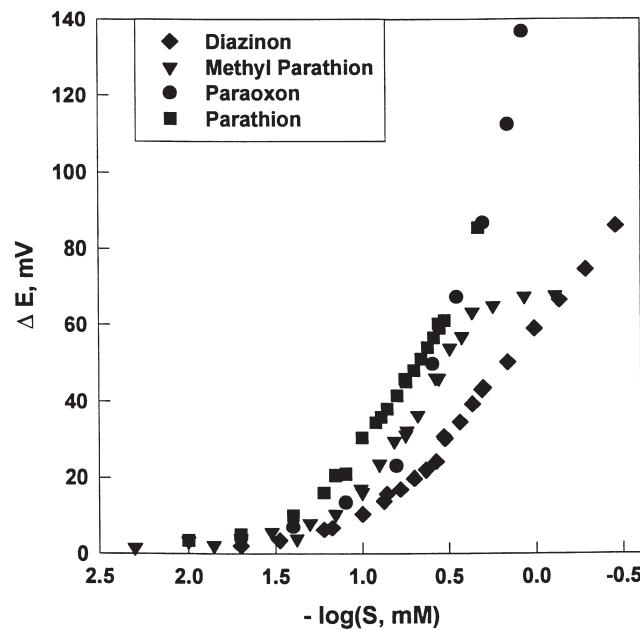


Fig. 6. Calibration plots for organophosphates: Conditions: 1 mM HEPES + 100 mM NaCl + 0.05 mM CoCl<sub>2</sub> (pH 8.5) 20°C; 500 IU of enzyme immobilized.

The 2  $\mu$ M lower detection limit (three times the standard deviation of the response obtained for a blank) of the present OPH-modified enzyme electrode for paraoxon is comparable to the OPH-based microbial biosensor (Rainina et al., 1996). This detection limit, however, is one to three orders of magnitude higher than for AChE-based biosensors (Paddle, 1996; Trojanowicz and Hitchman, 1996; Andres and Narayanaswamy, 1997; Danzer and Schwedt, 1996; Martorell et al., 1997; Palchetti et al., 1997). This will therefore limit the applicability of the present sensor for environmental monitoring to off-line analysis. For any such application of the present biosensor, off-line sample preparation involving solvent extraction and concentration will be necessary. The present enzyme electrode, however, will be ideal for: (1) on-line monitoring of detoxification processes for the treatment of wastewater generated during the production and consumption of the organophosphate-based pesticides and insecticides and disposal of organophosphate-based nerve agents; and (2) selectively monitoring only the organ-

ophosphate-based pesticides/neurotoxins. Theoretical models for enzyme electrode with non-reactive transducer predict that enzyme electrode sensitivity and detection limit can be improved by either lowering the enzyme  $K_M$  or increasing the bimolecular rate constant (Carr and Bowers, 1980; Eddowes, 1990). Thus, it is foreseeable that an enzyme electrode of higher sensitivity and lower detection limit will be realizable by lowering the  $K_M$  and/or increasing the bimolecular rate constant of OPH for the nerve agents to be analysed. The advancements in enzyme engineering have made these goals potentially achievable. One such example of site-directed mutagenesis of OPH in order to improve the rate of hydrolysis of the chemical warfare agent, soman, was recently reported (Lai et al., 1996; Mason et al., 1997).

### 3.6.2. Precision and accuracy of the enzyme electrode

The low relative standard deviations of 4.23% ( $n = 9$ ), 1.29% ( $n = 8$ ) and 3.54% ( $n = 7$ ), respectively, for paraoxon, methyl parathion and parathion, demonstrate the high precision of analysis. Additionally, a very low relative standard deviation of 5% ( $n = 4$ ) in the response of four enzyme electrodes prepared using 500 IU of OPH and tested in 1 mM HEPES with 100 mM NaCl (pH 8.5) at 20°C to 0.1 mM paraoxon demonstrates an excellent reproducibility from enzyme electrode-to-enzyme electrode.

Simulated samples representing the feed to a biological (enzymatic/microbial) biotransformation process (Caldwell and Raushel, 1991; Chen and Mulchandani, 1998) were analysed using the enzyme electrode and conventional enzymatic assay (based on the concentration of *p*-nitrophenol formed by enzymatic hydrolysis of the three tested pesticides). The good agreement (Fig. 7) between the two methods indicate the sensor is very accurate and reliable.

### 3.6.3. Selectivity

Unlike the AChE-based biosensors, the present enzyme electrode is highly specific for organophosphates. Other widely used pesticides, atrazine, sutan, sevin and simazine at 20  $\mu$ M concentrations do not interfere (Table 2). This is a significant benefit over the AChE-based biosensors, specially for the on-line monitoring of detoxification processes.

Table 1  
Analytical characteristics of OPH-modified potentiometric enzyme electrode

Compound	Maximum sensitivity, mV/decade concentration	Linear range corresponding to maximum sensitivity, mM	Detection limit, $\mu$ M
Paraoxon	150.44	0.15–0.7	2
Parathion	64.96	0.06–0.47	2
Methyl parathion	68.99	0.1–0.43	2
Diazinon	53.18	0.13–2.8	5

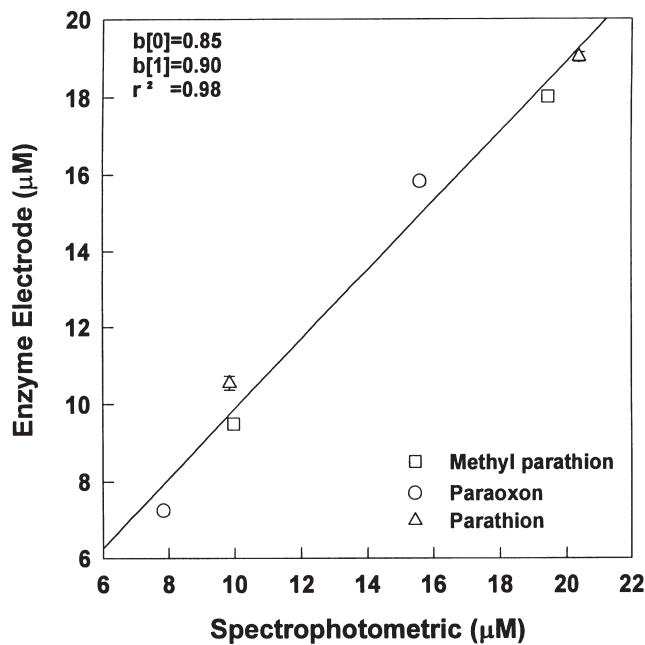


Fig. 7. Comparison of the organophosphate measurement by the enzyme electrode and enzyme assay technique. Results are average of three measurements.

Table 2  
Response of the OPH-modified potentiometric enzyme electrode to other pesticides

Pesticide	Concentration, mM	Response, mV
Atrazine	0.02	0
Simazine	0.02	0.1
Sevin	0.02	0.2
Sutan	0.02	0

### 3.6.4. Stability and response time of the enzyme electrode

For a sensor to be suitable for on-line process monitoring application, it should have excellent multiple use and long-term storage stability and a short response time. The long-term storage and the multiple use stability of the enzyme electrode was investigated by evaluating the response of the same electrode to paraoxon and storing it back at 4°C in pH 8.5, 1 mM HEPES plus 100 mM sodium chloride + 0.05 mM CoCl<sub>2</sub> buffer. As the results show (Fig. 8), the enzyme electrode was very stable, retaining over 95% of its original response, for the one month period during which it was used a total of 20 times. These results are in agreement with the OPH-based microbial biosensor developed by Rainina et al. (1996). In comparison, due to AChE inactivation every time the biosensor is exposed to organophosphates (Marty et al., 1992; Dzyadevich et al., 1994; La Rosa et al., 1994), AChE-based biosensors lost as much as 40% of the original response after as few as three repeated uses (Marty et al., 1992; La Rosa et al., 1994).

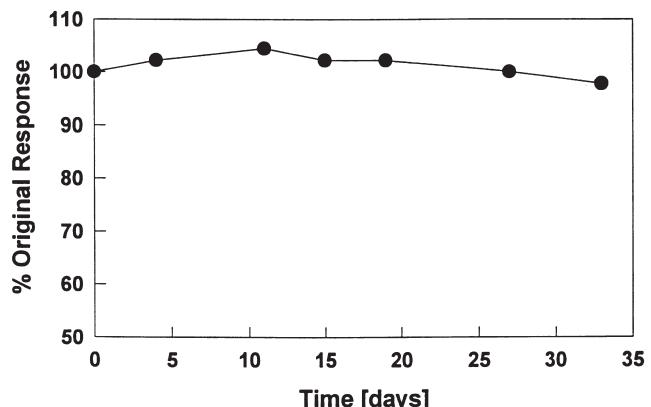


Fig. 8. Stability of the OPH-modified potentiometric electrode. Response of the electrode to 0.1 mM paraoxon in 1 mM HEPES + 100 mM NaCl + 0.05 mM CoCl<sub>2</sub> (pH 8.5) at 20°C. Enzyme amount: 500 IU.

The OPH-based enzyme electrode involves a simple single-step direct measurement. The time for analysing each sample in the steady-state mode (determined from the time required to achieve 90% of maximum response) was 10 min, while it was only 2 min in the kinetic mode (time to measure the initial slope of the mV vs. time response trace). These response times are a slight improvement over the OPH-based microbial biosensor (Rainina et al., 1996). AChE-based biosensors, in contrast, involve multiple steps (measurement of the initial AChE activity, incubation with the neurotoxin, determination of the inhibited enzyme activity and regeneration of enzyme activity) and indirect measurement requiring anywhere from 15 min (these biosensors are the disposable type where the enzyme reactivation step is excluded and is hence unsuitable for multiple use on-line process monitoring) to up to 5 h (Tran-Minh et al., 1990; Marty et al., 1992; Trettnak et al., 1993; La Rosa et al., 1994; Diehl-Faxon et al., 1996; Andres and Narayanaswamy, 1997; Palchetti et al., 1997).

## 4. Conclusions

In conclusion, an OPH-modified potentiometric enzyme electrode for the direct, rapid and selective measurement of organophosphate nerve agents was developed. Unlike the OPH-based microbial biosensor (Rainina et al., 1996), which requires specialized tools for cryoimmobilization, the construction of this enzyme electrode is very simple. The sensor had excellent stability, precision, accuracy, selectivity for organophosphate over other neurotoxins and short response time. These features make it a potential analytical tool ideal for the long-term monitoring of chemical and biological detoxification processes. Additionally, the enzyme electrode can potentially be used as a detector downstream

of any chromatographic separation to determine concentrations of individual organophosphate nerve agents.

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