

## Feature Article

# Organophosphorus Hydrolase-Based Amperometric Sensor: Modulation of Sensitivity and Substrate Selectivity

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## Abstract

The detection of organophosphate (OP) insecticides with nitrophenyl substituents is reported using an enzyme electrode composed of organophosphorus hydrolase (OPH) and albumin co-immobilized to a nylon net and attached to a carbon paste electrode. The mechanism for this biosensor involves catalysis of the OP followed by the electrochemical detection of the product, *p*-nitrophenol. The application of small amounts of mineral oil or silicon oil to the surface of the immobilized enzyme increased the stability and response signals of these sensors. In the case of silicon oil, the response for parathion was five times higher than without oil. In addition, the application of either mineral or silicon oil to the enzyme-coated membranes increased the relative selectivity of the sensor for parathion over paraoxon, most likely reflecting the partition of the substrates between the aqueous and oil phases. The enzyme activity was stabilized for more than two months by the application of either oil to the electrode surface. Compounds evaluated using this technique included parathion, EPN, fenitrothion, and paraoxon. The detection limits for parathion and paraoxon are 15 nM and 20 nM, respectively. These detection limits are substantially lower than those reported for optical assays (e.g.,  $\mu$ M range) and lower than those reported using several OPH electrodes (e.g., high nM range). Several characteristics that make this enzyme electrode attractive for further development as a potential field analytical method include; its simplicity and stability, as well as the relatively small amount of enzyme required (i.e., 3 International Units).

**Keywords:** Organophosphate, Insecticides, Enzyme electrode, Organophosphorus hydrolase

## 1. Introduction

Because organophosphate (OP) compounds are widely used in the U.S. and International agricultural industry, much attention has been focused on the development of the biosensors for the rapid and effective determination of these insecticides [1–6]. The high volume use of these compounds carries with it the potential for the contamination of the surface water, ground water and soil, and could lead to human or ecosystem exposures. Laboratory-based methods, which are commonly used for the assay of OP pesticides, include gas chromatography (GC), high performance liquid chromatography (HPLC), and capillary electrophoresis [7]. Although these methods are sensitive and specific, they are not particularly well suited for development for field applications.

The need for rapid and cost-effective field analytical methods for monitoring OP insecticides has resulted in the development of many techniques, a number of which are bioanalytical in nature. These bioanalytical methods are based primarily on cholinesterase inhibition [8] and immunoassay [9]. Enzyme-linked immunosorbent assays (ELISA) are quite sensitive but, like most immunoassays, target a single compound and require multiple steps. Cholinesterase-based enzyme inhibition assays are also well suited for screening applications. However, due to their irreversible

inhibition, they are not particularly well suited for process control monitoring applications which require rapid and repeated measurements.

OPH catalyzes the hydrolysis of a wide range of OP pesticides [10]. As a result of its versatility, this enzyme has been incorporated into a number of assays and sensors for detection of OP compounds and nerve agents [1–4, 11]. Because OPH-based assays respond to OP compounds as substrates rather than inhibitors, these assays are reversible as compared to methods that use enzyme inhibition. As a result of these characteristics, OPH-based biosensors show considerable potential for development in applications that require repetitive analysis. Assay formats used with this enzyme have involved potentiometric [12] or fluorescence [1] measurement of local pH changes as well as amperometric measurement of electroactive enzyme products [4]. In the cases of amperometric measurement, electrochemically active products such as para-nitrophenol are typically measured. Immobilization strategies that have been reported for OPH electrodes include the use of polyethylenimine on carbon ink electrodes [2], Nafion on carbon ink [4], or Nafion on carbon paste [3].

This article reports the amperometric detection of parathion, EPN, paraoxon and fenitrothion using an enzyme electrode composed of OPH immobilized on a nylon net in direct contact with a carbon paste electrode. One of the

advantages of using the nylon net for enzyme immobilization is the minimal amount of OPH required which is about 100 times less than optical [1] or electrochemical [3] sensor methods. It is further reported that the saturation of the enzyme-coated nylon net with mineral or silicon oil improves the stability, enhances the sensitivity, and changes the selectivity of the enzyme electrode. In addition, the low detection limits for the herein reported enzyme sensor represent a significant improvement over previously reported OPH biosensor methods [1, 3, 4].

## 2. Experimental

### 2.1. Materials

Parathion, EPN, fenitrothion, and paraoxon were obtained from Chem Service (West Chester, PA). *p*-Nitrophenol, albumin, mineral oil (light white oil) and 2-[*N*-cyclohexylamino]ethanesulfonic acid (CHES) were purchased from Sigma, and silicon oil DC 200 (100 mPa s at 25 °C) was from Fluka. Nylon net (200 mesh) was obtained from Ted Pella, Inc. (Redding, CA). Graphite powder (1–2 μm) was purchased from Aldrich. Organophosphorus hydrolase (2.78 U/10 μL) was purified as previously reported [12]. All other compounds and solvents used were of reagent grade.

### 2.2. Electrode

The carbon paste was prepared by mixing 600 mg of graphite powder with 400 mg of mineral oil [13]. The paste was firmly packed into the electrode cavity and the surface smoothed using a weighing paper. OPH was immobilized on the nylon net through crosslinking to albumin. An aliquot (5 μL) of 3% albumin was applied to the surface of the net. After drying at room temperature, 10 μL of OPH solution was dispensed onto the surface followed by another 5 μL of 3% albumin solution. Crosslinking was initiated by exposure to glutaraldehyde vapor. This was accomplished by placement of the protein-coated net above the solution in 25 mL sealed bottles containing 60 μL of 5% glutaraldehyde for 5 hours.

### 2.3. Assay Procedure

Enzyme activity was determined by measuring the absorbance of *p*-nitrophenol (at 400 nm) released from paraoxon [1]. The initial concentration of paraoxon was 1 mM in 50 mM CHES buffer at pH 9.0. The activity for 10 μL of enzyme solution was 2.78 units.

Amperometric determinations were performed using a three electrode system (enzyme electrode, Ag/AgCl reference, Pt counter). The potential applied was +0.85 V. Experiments were run in a batch mode. After the background signal reached a steady-state, aliquots of samples and standards were added to a stirred 0.1 M phosphate

buffer solution (pH 8.5). Primary stock solutions were as follows: paraoxon (5 mM aqueous), parathion (5 mM in water:methanol; 2:1). EPN and fenitrothion stock solutions (0.01 mM) were prepared in 10% methanol by dilution of the corresponding 5 mM stock solution in methanol. All the stock solutions were stored at 4 °C. The degree of hydrolysis was routinely determined prior to the assay by the measuring the absorbance of *p*-nitrophenol at 400 nm using spectrophotometer.

## 3. Results and Discussion

The interface of the OPH-containing nylon net to the carbon paste electrode allowed the electrochemical detection of OP compounds that release *p*-nitrophenol leaving groups. This was accomplished by measuring the oxidation of *p*-nitrophenol (leaving group for parathion, EPN and paraoxon) or 3-methyl-*p*-nitrophenol (leaving group for fenitrothion). The amperometric response of *p*-nitrophenol at a carbon paste electrode is shown in Figure 1. The calibration plot was linear over the range of 2–40 nM. These results are similar to those reported by Sacks et al. [2] using a carbon electrode incorporated into a flow injection system.

The carbon paste electrode interfaced to the enzyme-coated nylon net responded to parathion (the OPH substrate) in a concentration-dependent manner (Fig. 2). When BSA alone was immobilized to the nylon net, there was no response to OP compounds. The calibration plot for the enzyme electrode was linear up to about 180 nM. Addition of mineral or silicon oil to the enzyme-coated nylon net resulted in an increased response to parathion (Fig. 2). In particular, the use of silicon oil significantly increased the signal response to about five times that of the enzyme-coated net without oil. Similar to the effects that we report for the OPH electrode, organic environments have

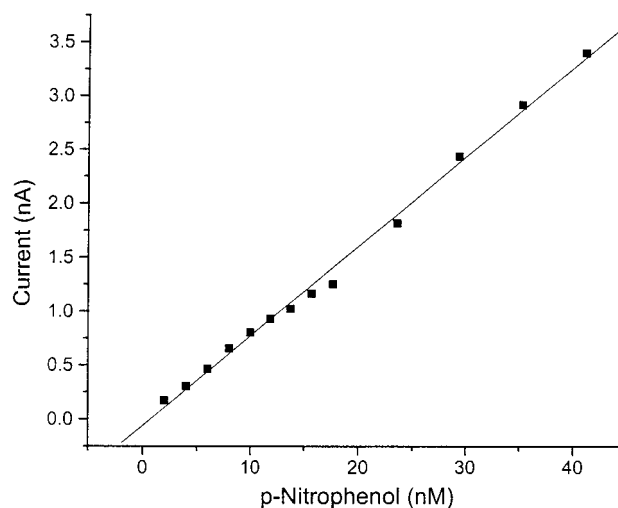


Fig. 1. Calibration of *p*-nitrophenol using carbon paste electrode. The potential vs 0.85 V (vs. Ag/AgCl reference) and the buffer was 0.1 M phosphate pH 7.0.

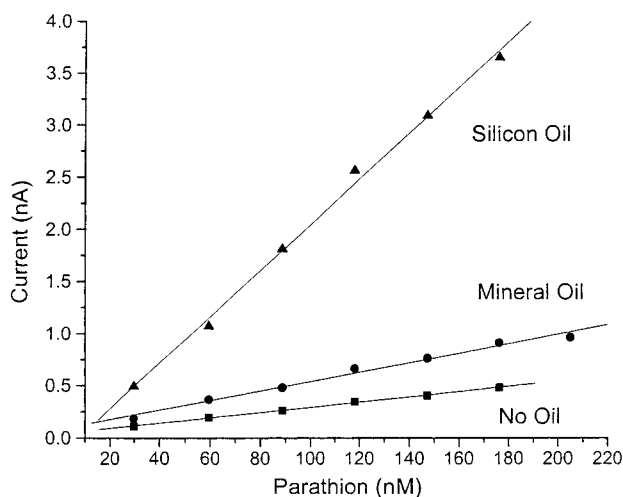


Fig. 2. Effect of silicon oil and mineral oil on OPH/nylon net/carbon paste electrode response to parathion. Experimental conditions were as described in Figure 1. Stable oil interfaces were established by saturating the nylon net.

been shown to affect the responses of several enzyme electrodes. For example, tyrosinase-containing carbon paste electrodes are sensitive to the effects of organic binders [13, 14]. The herein reported data, however, characterize, for the first time, the effect of an organic environment on the response of an OPH enzyme electrode.

Incorporation of silicon oil into the enzyme-coated nylon net increased the signal response for both parathion and paraoxon (Table 1). The use of mineral oil also increased the signal responses to parathion, however, it decreased the response to paraoxon. Further analysis of the signal response slopes indicated that for the immobilized enzyme without oil, paraoxon showed a greater response than parathion, yielding a response slope ratio (parathion/paraoxon) of 0.6. In the case of oil-treated electrodes, however, the ratios were greater than 1.0 for both mineral oil (1.4) and silicon oil (1.8). These results suggest that partitioning of the substrates between the aqueous and oil phases plays a significant role in the observed results. More specifically, the increase in the biosensor response ratio of parathion to paraoxon upon addition of mineral oil or silicon oil is likely due to increased partitioning of the less polar parathion and decreased partitioning of the more polar paraoxon into the oil phase of the saturated nylon net. Partitioning effects for substrates in other enzyme electrodes such as for the tyrosinase carbon paste electrodes constructed using various pasting liquids have also been reported [14]. For

previously reported tyrosinase electrode systems, a more favorable nonpolar environment provided by the carbon paste electrode appeared to favor the enhancement of sensitivity for more nonpolar substrates [14].

The silicon oil-treated enzyme electrode was further characterized with respect to several OP compounds (Fig. 3). Parathion showed the greatest response followed by paraoxon, EPN and fenitrothion. The relative responses of the OPH electrode to these substrates may be influenced by two main factors; changes in the relative partitioning of parathion and paraoxon in the organic phase and a relative change in the  $K_{m,app}$  for each of these substrates upon immobilization of the enzyme. Because immobilization of OPH has been reported to increase the  $K_m$  values for these substrates by 3–4 times [15], we suggest that the observed differences in electrode response is due to changes in relative partitioning.

## 5. Conclusions

We report a new configuration for an OPH electrode biosensor consisting of a carbon paste electrode with an attached enzyme-coated nylon net. The biosensor is sensitive and durable. The immobilization of OPH on the net requires less enzyme compared to directly mixing the

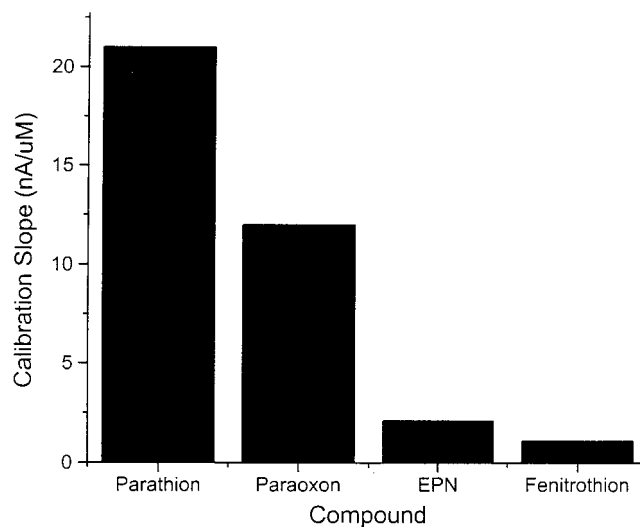


Fig. 3. Enzyme electrode response to parathion, paraoxon, EPN and fenitrothion. Calibration slopes for the silicon oil-treated enzyme electrode were determined by least squares analysis of the calibration plots.

Table 1. Effect of mineral oil and silicon oil on OPH electrode selectivity.

OPH/Nylon Net	Parathion (nA/μM ± SEM)	Paraoxon (nA/μM ± SEM)	Ratio (Parathion/Paraoxon)
No Oil	4.9 ± 0.6	8.1 ± 0.9	0.6
Mineral Oil	6.1 ± 0.9	4.5 ± 0.7	1.4
Silicon Oil	21.0 ± 0.1	12.0 ± 0.8	1.8

enzyme into a polymer used to coat carbon paste [3] or carbon ink [4] electrodes. The enzyme electrode can be prepared simply by pressing the OPH membrane against the carbon paste electrode. In addition, the biosensor response is significantly enhanced by saturation of the nylon net with silicon oil. This enhancement is selective for parathion over paraoxon.

## 6. Acknowledgements

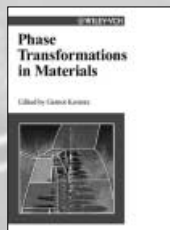
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