*Sensors and Materials*, Vol. 8, No. 3 (1996) 161–167
MYU Tokyo

S & M 0233

# Comparison of Two Transduction Modes for Design of Microbiosensors Applicable to Detection of Pesticides

Nicole Jaffrezic-Renault*, Claude Martelet, Paul Cléchet, André-Marie Nyamsi Hendji, Alexander A. Shul'ga[1]**, Sergei V. Dzyadevitch[1], Larissa I. Netchiporuk[1] and Alexei. P. Soldatkin[2]

Laboratory of Interfacial Physicochemistry,
URA CNRS 404, Ecole Centrale de Lyon, BP 163, 69131 Ecully Cedex, France
[1] Semiconductor Physics Department, Sector of Bioelectronics, Kiev University,
P. O. Box 152, Kiev 252001, Ukraine
[2] Institute of Molecular Biology and Genetics,
Academy of Sciences of the Ukrainian
SSR., 150, Zabolotny St., Kiev 252143, Ukraine

(Received October 31, 1994; accepted July 2, 1995)

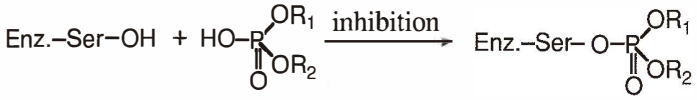
In this work, the performances of two simple biosensor designs, respectively based on potentiometric and conductimetric transduction modes, have been compared for the detection of organophosphorous pesticides. The analytical characteristics are very similar for the two types of microbiosensors and for the two enzymatic systems. The dynamic ranges are $10^{-12}$M – $10^{-7}$M for DFP and $10^{-7}$M – $10^{-4}$M for paraoxon-methyl and trichlorfon. The detection limits are $10^{-12}$M for DFP and $10^{-7}$M for paraoxon-methyl and trichlorfon. The recovery of the enzymatic activity through treatment with a PAM-2 reagent requires at least one hour. Since these microsensors are based on an unselective enzyme activity inhibition, they can be used as disposable systems for monitoring the overall toxic contents of the assayed samples.

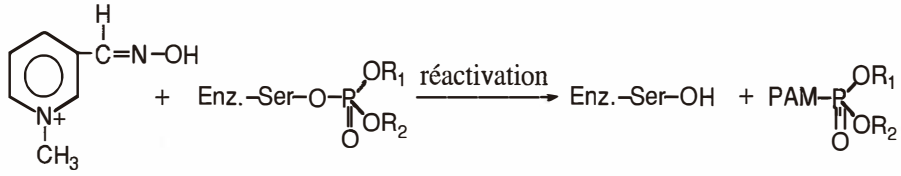
* To whom correspondence should be addressed.
** Current address: Institut für Chemo- und Biosensorik e.v., Mendelstrasse 11, D-48149 Münster, Germany

## 1. Introduction

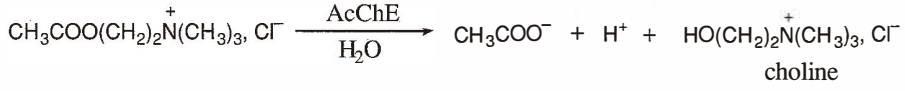
Since pesticides are among the most toxic products of the chemical industry, in Europe, regulations limit their maximum concentration in drinking water to 1 $\mu g \cdot ml^{-1}$. Therefore, a strong demand for disposable, low cost and simple monitoring devices exists.

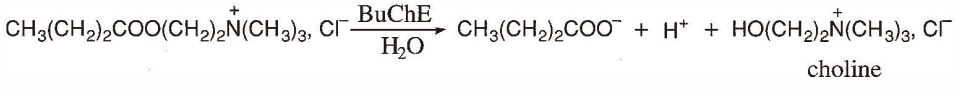
In this work, the performances of two simple biosensor designs, respectively based on potentiometric and a conductimetric transduction modes, have been compared for the detection of organophosphorous pesticides. The basic action of these compounds is associated with their ability to inhibit acetylcholinesterase (AcChE) and butyrylcholinesterase (BuChE) by phosphorylating the serine OH group in the active site of the enzyme, the reactivation of the enzyme being obtained after a treatment with PAM-2 reagent (pyridin-2 aldoxime methiodide).




Normally, AcChE and BuChE hydrolyze acetylcholine chloride and butyrylcholine chloride according to the following reactions.




One of the resulting products, $H^+$, can be easily detected either by the potentiometric transducer, pH-ISFET (ion sensitive field effect transistor for pH detection), or by the

conductimetric transducer through the variation in conductivity induced in the enzymatic membrane. The decrease in the concentration of the enzymatic reaction product caused by enzyme inhibition can be related to pesticide concentration.

## 2. Materials and Methods

### 2.1 *Enzymes and reagents*

AcChE (true cholinesterase, EC 3.1.1.7.) from electric eel, type VI-S, 225 $U \cdot mg^{-1}$; butyryl cholinesterase (BuChE) (pseudo-cholinesterase, EC 3.1.1.8) from horse serum, 255 $U \cdot mg^{-1}$, acetylcholine chloride (AcChCl) and butyrylcholine chloride (BuChCl) were obtained from Sigma (La Verpillière, France), bovine serum albumin from Boehringer Mannheim (Meylan, France) and 25% glutaraldehyde (GA) solution from Fluka (Buchs, Switzerland).

Diisopropyl fluorophosphate (DFP), dimethyl(2,2,2-trichloro-1-hydroxyethyl)phosphate (trichlorfon) were purchased from Sigma, and paraoxon-methyl and paraoxon-ethyl from Riedel-de-Haën (Seelze, Germany). Phosphate buffer ($KH_2PO_4$) and PBS were provided by Merck (Darmstadt, Germany) and Sigma, respectively. Other reagents were of analytical grade.

### 2.2 *Potentiometric transduction*

For the potentiometric transduction mode, two identical pH FETs were micromachined on the same p-Si wafer (cf. Fig. 1). This sensor chip was fixed on a fused silica support, wired to aluminum contacts, and then encapsulated into an epoxy resin. These devices were produced at the Microdevices Research Institute (Kiev, Ukraine). Details on this design and on the differential measurement setup can be found in ref. 1.

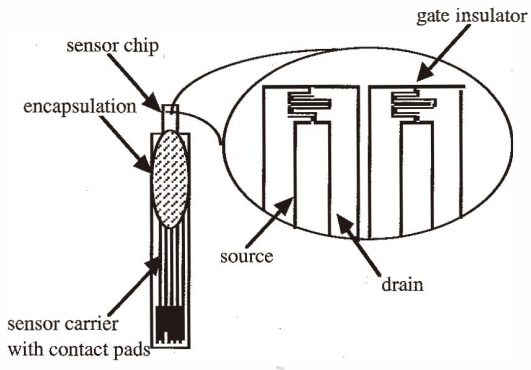



Fig. 1. Potentiometric transducer design.

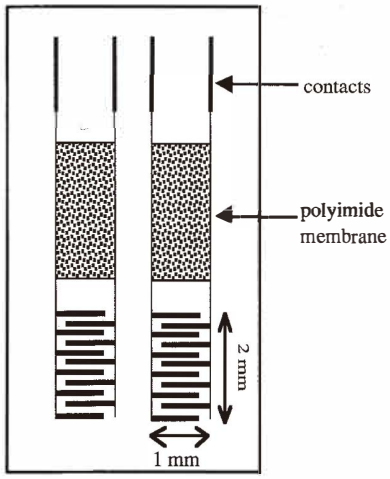



Fig. 2. Conductimetric transducer design.

### 2.3 *Conductimetric transduction*

In the case of the conductimetric transduction mode, two identical pairs of gold-interdigitated electrodes (thickness 0.5 mm, dimensions 5 mm × 40 mm) were fabricated by vacuum deposition on a ceramic substrate (fused aluminum oxide) (cf. Fig. 2). An intermediate layer of chromium (0.1 $\mu$m thick) was used for better gold adhesion. Each finger of the electrode was 70 $\mu$m wide and about 1 mm long, with 70 $\mu$m spacing between fingers of the electrodes in the pair. The sensing area of each electrode pair was about 1 mm × 1.5 mm; in order to define it, the central part of the chip was covered with a polymerized polyimide layer. These devices were produced at the Microdevices Research Institute (Kiev, Ukraine). Further information on the device and on the differential measurement setup can be found in ref. 2.

### 2.4 *Enzyme immobilization*

The sensitive enzymatic membranes were prepared by enzyme cross-linking with bovine serum albumin (BSA) and deposited onto the sensing part of the chip, the reference part being coated with a membrane where the enzyme was replaced by BSA. The method used was previously reported.[3,4] The average enzyme quantity in the deposit was equal to around 10 $\mu$g.

### 2.5 *Measurement of enzyme activity, inhibition and reactivation*

AcChE and BuChE membranes were tested using acetyl- and butyrylcholine chloride as substrates. The decrease of substrate steady-state response caused by exposure of the biosensor to pesticides (DFP, trichlorfon, paraoxon-methyl and paraoxon-ethyl) was used to estimate the enzyme inhibition. The optimized parameters for potentiometric[1] and conductimetric[2] measurement procedures were previously determined.

## 4. Results and Discussion

Figures 3 and 4 show typical curves of the inhibition effect obtained with DFP on AcChE biosensors, respectively for ENFET and the conductimetric transducer.

Analytical characteristics of these two types of microbiosensors for the determination of pesticides are respectively given in Tables 1 and 2 for ENFET and the conductimetric transducer. Their analytical characteristics are very similar with respect to the dynamic range and the detection limit. The slight discrepancies can be explained by the difficulty in obtaining enzymatic membranes with highly reproducible thickness, cross-linking rate and enzyme content.

Experiments on the recovery of enzymatic activity have been conducted, using biosensors treated with a PAM-2 reagent (pyridin-2 aldoxime methiodide); however, such regeneration requires at least one hour and is incomplete for high inhibition levels and long exposure time.

## 5. Conclusion

Performances of these two sensor designs are not significantly different in the detection of organophosphorus pesticides in aqueous solutions.

Since these microsensors are based on an unselective enzyme activity inhibition they can be used as disposable systems for monitoring the overall toxic contents of the assayed samples. These devices could be directly applied to environmental control in alarm systems.

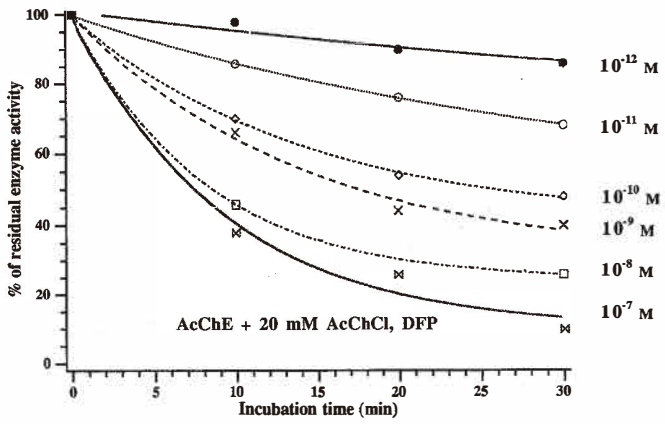



Fig. 3. Inhibition effect on the AcChE ENFET as a function of the incubation time and of the concentration of DFP.

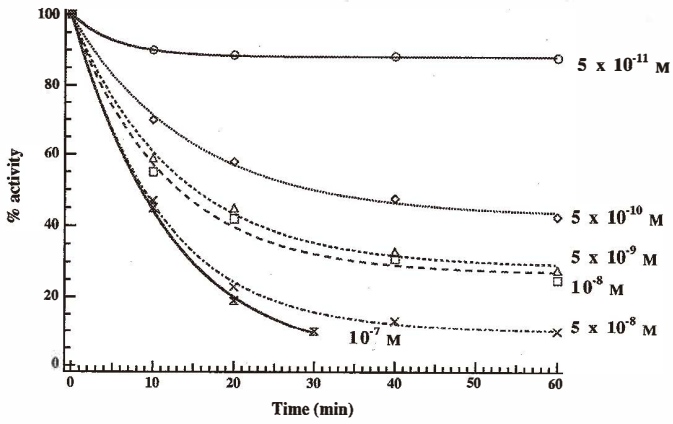



Fig. 4. Inhibition effect on the AcChE conductimetric transducer as a function of the incubation time and of the concentration of DFP.

Table 1
Analytical characteristics of the ENFET biosensors for the determination of organophosphorous pesticides.

| Pesticide | AcChE membranes | | | BuChE membranes | | |
|---|---|---|---|---|---|---|
| | Detection limit | | Dynamic range, M | Detection limit | | Dynamic range, M |
| | $\mu g \cdot l^{-1}$ | M | | $\mu g \cdot l^{-1}$ | M | |
| DFP | 0.0002 | $10^{-12}$ | $10^{-12} - 10^{-7}$ | 0.0002 | $10^{-12}$ | $10^{-12} - 10^{-8}$ |
| Paraoxon-methyl | 25 | $10^{-7}$ | $10^{-7} - 10^{-4}$ | 250 | $10^{-6}$ | $10^{-6} - 10^{-4}$ |
| Trichlorfon | 24 | $10^{-7}$ | $10^{-7} - 10^{-4}$ | 240 | $10^{-6}$ | $10^{-6} - 10^{-3}$ |

Table 2
Analytical characteristics of the conductimetric biosensors for the determination of organophosphorous pesticides.

| Pesticide | AcChE membranes | | | BuChE membranes | | |
|---|---|---|---|---|---|---|
| | Detection limit | | Dynamic range, M | Detection limit | | Dynamic range, M |
| | $\mu g \cdot l^{-1}$ | M | | $\mu g \cdot l^{-1}$ | M | |
| DFP | 0.004 | $5 \times 10^{-11}$ | $5 \times 10^{-11} - 10^{-7}$ | 0.004 | $5 \times 10^{-11}$ | $5 \times 10^{-11} - 10^{-8}$ |
| Paraoxon-methyl | 250 | $10^{-6}$ | $10^{-6} - 10^{-3}$ | 250 | $10^{-6}$ | $10^{-6} - 10^{-4}$ |
| Paraoxon-ethyl | 250 | $10^{-6}$ | $10^{-6} - 10^{-4}$ | 250 | $10^{-6}$ | $10^{-6} - 10^{-4}$ |
| Trichlorfon | 24 | $10^{-7}$ | $10^{-7} - 10^{-4}$ | 24 | $10^{-7}$ | $10^{-7} - 10^{-4}$ |

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