Disposable Potentiometric Enzyme Sensor for Direct Determination of Organophosphorus Insecticides

Authors:
Gaberlein, S., Knoll, M., Spener, F., Zaborosch, C.

Reviewer:
Bax Smith, B.Sc. (Chemistry), B.Eng. (Electrical Engineering), M.Eng. Candidate (Robotics)
Outline

- Development of the Disposable Sensor for OP
  - Why?, Preparation, Structure, Double Matrix Membranes (DMM)

- OP Disposable Sensor Performance
  - Effect of enzyme loading, starting pH, buffer concentration and temperature
  - Calibration Results
  - Specificity, reproducibility, precision and long term storage stability

- Advantages/DisAvantages
Development of a Disposable Sensor for OP
Why?

- Organophosphorus Compounds are very toxic and are widely used in insecticides
  - Results in irreversible inhibition of acetylcholinesterase (AChE) in both target organisms and non-target organisms (mammals)

- Detection Techniques
  - GC, LC and TLC work well but are time consuming and expensive
  - Biosensors based on inhibition of cholinesterase activity usually require long incubation times and long regeneration times
The Solution

- A Biosensor based on the hydrolytic cleavage reaction of organophosphorus hydrolase (OPH)
  - OPH hydrolyzes a wide range of OP esters
  - Hydrolysis of one OP molecule releases 2 protons from the products
Preparation

- OPH isolated from E. coli DH5α cells
- Construct Sensor
- Double Matrix Membrane (DMM)
  - 1% N,N-dioctadecylmethylamine (H+-ionophore), 67% bis(2-ethylhexyl)sebacate, 0.3% sodium tetr phenylborate, 31.7% PVC dissolved in tetrahydrofuran and cyclohex anone
- Enzyme Sensors
  - Poly(carbamoyl sulfonate) PCS + OPH drops polymerize adjacent to DMM
- Dip in buffer with 100mM Sodium Chloride to fill reference electrode filter paper
Structure - Side

- Heat sealing film (150 µm)
- Contact ISE
- Preperforated heat sealing film
- Conducting line (silver carbon ink)
- PCS + OPH
- Filter paper
- H⁺-sensitive membrane mixture
- Conducting line (with Ag/AgCl)
- Contact reference electrode
- Heat sealing film
- Filter paper
Structure – Front/Back

Diagram:
- Heat sealing film
- Contact ISE
- Conducting line (silver carbon ink)
- Preperforated heat sealing film
- PCS + OPH
- Double matrix membrane (DMM)
- 33 mm
- 8 mm
- Opening for measuring solution
- Heat sealing film
- Conducting line (with Ag/AgCl)
- Contact reference electrode
- Filter paper
OP Disposable Sensor Performance
pH Calibration

- pH calibration of 10 electrodes gave a slope with mean value 55.2 mV/decade (SD = 1.9%) over pH range 11 to 6
  - Acceptable value is 48, Nernst value is 59
Effect of Enzyme Loading

Fig. 2 Effect of OPH loading on initial hydrolytic rates of the enzyme sensor measuring paraoxon (0.1 mM) in 1.0 mM HEPES buffer (pH 9.3), 100 mM NaCl, 37 °C (mean values ± s, n = 3).
Effect of Buffer Concentration

Fig. 4  Effect of the buffer concentration on the initial hydrolytic rate of enzyme sensors containing 250 U OPH (sensor age: 1 d) measuring paraoxon (0.1 mM) in HEPES buffer (pH 9.3) at 37 °C (mean values ± s, n = 3).
Calibrations with OP Compounds

Table 1  Analytical characteristics of disposable enzyme sensors containing 500 U OPH (sensor age: 2 d)

<table>
<thead>
<tr>
<th>OP compound</th>
<th>Maximum sensitivity/mV decade$^{-1}$ OP concentration</th>
<th>Linear range corresponding to maximum sensitivity/mM</th>
<th>Lower detection limit$/\mu$M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraoxon</td>
<td>98</td>
<td>0.05–0.4</td>
<td>5</td>
</tr>
<tr>
<td>Diazinon</td>
<td>61</td>
<td>0.05–0.35</td>
<td>5</td>
</tr>
<tr>
<td>Parathion</td>
<td>37.7</td>
<td>0.007–0.05</td>
<td>5</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>24.6</td>
<td>0.01–0.15</td>
<td>5</td>
</tr>
</tbody>
</table>

*$^a$ Defined as three times the background noise of the electrode.

Fig. 5  Calibration plots for organophosphorus compounds chlorpyrifos (○), diazinon (□), paraoxon (▼) and parathion (▲) with enzyme sensors containing 500 U OPH (sensor age 2 d). Measuring buffer was 1.0 mM HEPES (pH 9.3), 100 mM NaCl at 37 °C. Errors of sensor values were below symbol size ($n = 3$). OP compounds were applied in methanol stock solutions. Methanol concentrations did not exceed 2.5%.
Specificity

Table 2  Specificity of disposable enzyme sensors (500 U OPH, sensor age: 3 d). Investigation of the influence of various substances on the response of enzyme sensors in HEPES buffer (1.0 mM, pH 9.3), 100 mM NaCl at 37°C (mean values ± s, n = 3)

<table>
<thead>
<tr>
<th>Substance</th>
<th>ΔΕ/mV</th>
<th>Δt/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraoxon</td>
<td>40.0 ± 0.5</td>
<td>55 ± 0</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.1 ± 0</td>
<td></td>
</tr>
<tr>
<td>Glucose + paraoxon</td>
<td>38.2 ± 1.2</td>
<td>55 ± 0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Sucrose + paraoxon</td>
<td>41.2 ± 0.7</td>
<td>65 ± 0</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Lactose + paraoxon</td>
<td>40.4 ± 0.8</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Maltose</td>
<td>5.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Maltose + paraoxon</td>
<td>40.4 ± 0.8</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Acetate</td>
<td>2.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Acetate + paraoxon</td>
<td>53.0 ± 4.0</td>
<td>115 ± 5</td>
</tr>
<tr>
<td>Gluconate</td>
<td>5.0 ± 0</td>
<td></td>
</tr>
<tr>
<td>Gluconate + paraoxon</td>
<td>41.0 ± 0.5</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>5.0 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Pyruvate + paraoxon</td>
<td>40.7 ± 1.2</td>
<td>110 ± 0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Glycerol + paraoxon</td>
<td>39.5 ± 1.0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Humic acid</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Humic acid + paraoxon</td>
<td>39.5 ± 1.5</td>
<td>260 ± 15</td>
</tr>
</tbody>
</table>

a Concentrations: paraoxon = 0.1 mM, humic acid = 20 mg l⁻¹, all other substances 15 mM.
Reproducibility, Precision and Long Term Storage Stability

- Reproducibility: $E$ values for 10 sensors had SD of 1.1%
- Precision: repeated use of one sensor had SD of 0.75% ($n=5$)
Advantages/Disadvantages

- Fast
- Inexpensive
- Easy to handle

- Higher detection limits (uM vs nM)
- Very sensitive to buffer concentration
Conclusion

- Very well suited to sewage and subsoil water samples where the content of buffering substances are low