DEVELOPMENT OF PEPTIDE NANOTUBE-MODIFIED BIOSENSORS FOR GAS-PHASE ORGANOPHOSPHATE DETECTION

THESIS

Peter A. Baker, Major, USMC

AFIT-ENV-13-M-35

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

DISTRIBUTION STATEMENT A
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED
The views expressed in this thesis are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the United States Government. This material is declared a work of the U.S. Government and is not subject to copyright protection in the United States.
DEVELOPMENT OF PEPTIDE NANOTUBE-MODIFIED BIOSENSORS FOR GAS-PHASE ORGANOPHOSPHATE DETECTION

THESIS

Presented to the Faculty
Department of Systems Engineering and Management
Graduate School of Engineering and Management
Air Force Institute of Technology
Air University
Air Education and Training Command

In Partial Fulfillment of the Requirements for the
Degree of Master of Science in Environmental Engineering

Peter A. Baker, BS
Major, USMC

March 2013

DISTRIBUTION STATEMENT A
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED
DEVELOPMENT OF PEPTIDE NANOTUBE-MODIFIED BIOSENSORS FOR GAS-PHASE ORGANOPHOSPHATE DETECTION

Peter A. Baker
Major, USMC

Approved:

Mark Goltz, Ph.D., Lt Col, USAF (Ret) (Chairman)  

Dong-Shik Kim, Ph.D. (Member)  

Amanda Schrand, Ph.D (Member)
Abstract

Vapor-phase detection of the model organophosphate (OP) malathion was achieved using enzymes encapsulated in peptide nanotubes and attached to gold screen printed electrodes. The peptide nanotubes had horseradish peroxidase (HRP) encapsulated inside and both acetylthiocholine (ASCh) and acetylcholinesterase (AChE) coated on the outside. ASCh hydrolysis, which produces thiocholine, was catalyzed by the AChE. The thiocholine was then oxidized by the electrode to produce a signal which could be measured by a cyclic voltammeter. HRP catalyzes the oxidation reaction, enabling a faster and stronger response with a much smaller sample that would otherwise be required. This response was inhibited in the presence of malathion vapor, with the extent of inhibition proportional to the malathion concentration down to the detection limit of the biosensor.

This study first established methods to consistently reproduce known concentrations of malathion vapor. Once the vapor concentration was established, peptide nanotube-modified gold screen printed electrodes were used to detect OP vapor. The nanotube-modified electrodes were first exposed to AChE, then to ASCh, and finally inserted into an airtight vial with a known concentration of malathion. Cyclic voltammograms were taken at each step of the process to monitor the changes in activity. The biosensor response was inhibited by OP concentrations as low as 12 ppbv, which is less than half the lethal limit of 30 ppbv for VX.
This research demonstrates the ability to use nano-modified biosensors for the detection of organophosphate vapor, an important development in countering weaponized organophosphate nerve agents and detecting commercially used pesticides.
Acknowledgments

I would like to thank my thesis advisor, Dr. Mark Goltz for offering me the opportunity to research this topic and for his valiant attempts to teach me about the fate and transport of chemicals in environmental media such as air, water, and soil. I would like to thank Dr. Dong-Shik Kim for sharing his vast knowledge and experience with biosensor research with me and offering me guidance throughout this research process. I would like to thank Dr. Amanda Schrand for serving on my committee and offering her expertise on a wide variety of nanotechnologies. I would also like to thank Dr. Daniel Felker for his assistance, expertise, ingenuity, and patience in the lab as we invented the wheel for this research.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>iv</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>viii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>Glossary of Acronyms</td>
<td>x</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>3</td>
</tr>
<tr>
<td>Biosensors</td>
<td>6</td>
</tr>
<tr>
<td>Cholinesterase biosensors</td>
<td>7</td>
</tr>
<tr>
<td>Nanotubes</td>
<td>9</td>
</tr>
<tr>
<td>III. Scholarly Article</td>
<td>12</td>
</tr>
<tr>
<td>Abstract</td>
<td>12</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>13</td>
</tr>
<tr>
<td>2. Materials and Methods</td>
<td>16</td>
</tr>
<tr>
<td>3. Results and Discussion</td>
<td>22</td>
</tr>
<tr>
<td>4. Conclusions</td>
<td>28</td>
</tr>
<tr>
<td>5. References:</td>
<td>29</td>
</tr>
<tr>
<td>IV. Conclusion</td>
<td>30</td>
</tr>
<tr>
<td>Additional Results and Discussion</td>
<td>30</td>
</tr>
<tr>
<td>Further Research</td>
<td>32</td>
</tr>
<tr>
<td>Bibliography</td>
<td>33</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.</td>
<td>Organophosphates (ChemDB Portal, 2004)</td>
<td>4</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>Normal acetylcholine hydrolysis (ATSDR, 2010)</td>
<td>5</td>
</tr>
<tr>
<td>Figure 3.</td>
<td>Inhibition of AChE catalysis by OP (ATSDR, 2010)</td>
<td>5</td>
</tr>
<tr>
<td>Figure 4.</td>
<td>Electrode Construction</td>
<td>15</td>
</tr>
<tr>
<td>Figure 5.</td>
<td>Reaction between ASCh and AChE</td>
<td>23</td>
</tr>
<tr>
<td>Figure 6.</td>
<td>Current inhibition in either nitrogen gas (6a) or 25 ppbv malathion vapor (6b)</td>
<td>25</td>
</tr>
<tr>
<td>Figure 7.</td>
<td>Results of the Longevity Test</td>
<td>26</td>
</tr>
<tr>
<td>Figure 8.</td>
<td>Biosensor response vs. concentration (error bars indicate standard deviation of replicates)</td>
<td>27</td>
</tr>
<tr>
<td>Figure 9.</td>
<td>Biosensor response vs. concentration (error bars indicate standard deviation of replicates) above detection limit</td>
<td>27</td>
</tr>
</tbody>
</table>
List of Tables

Table 1. Important events in the development of biosensors (Taylor and Schultz, 1996) 7

Table 2. Longevity experiment setup........................................................................................................... 20

Table 3. Tests of weaponized nerve agents .................................................................................................... 31
### Glossary of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>ASCh</td>
<td>Acetylthiocholine</td>
</tr>
<tr>
<td>BCh</td>
<td>Butyrylthiocholine</td>
</tr>
<tr>
<td>BChE</td>
<td>Butyrylcholinesterase</td>
</tr>
<tr>
<td>H-Phe-Phe-OH</td>
<td>DI-L-Phenylalanine</td>
</tr>
<tr>
<td>HAPSITE</td>
<td>Hazardous Air Pollutants on Site</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish Peroxidase</td>
</tr>
<tr>
<td>OP</td>
<td>Organophosphate</td>
</tr>
<tr>
<td>PNT</td>
<td>Peptide Nanotube</td>
</tr>
<tr>
<td>ppbv</td>
<td>Parts Per Billion by Volume</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts Per Million</td>
</tr>
<tr>
<td>SPE</td>
<td>Screen-Printed Electrode</td>
</tr>
</tbody>
</table>
DEVELOPMENT OF PEPTIDE NANOTUBE-MODIFIED BIOSENSORS FOR GAS-PHASE ORGANOPHOSPHATE DETECTION

I. Introduction

Since their inception in 1937, chemical warfare nerve agents such as sarin and VX have posed one of the deadliest threats in both conventional warfare and terrorist tactics (Upadhyayula, 2011). One fifty-pound warhead of VX aerosolized by a relatively small explosion could kill approximately 2.5 million people (Ven Der Leun, 1972). These organophosphate (OP) nerve agents irreversibly bind to acetylcholinesterase (AChE) in the central nervous system. AChE is an enzyme that is produced to hydrolyze acetylcholine (ACh). ACh stimulates muscles, and when an OP agent has irreversibly bound to AChE, ACh accumulates, resulting in continuous stimulation of muscle groups. In particular, the diaphragm cannot relax, causing death by suffocation within minutes (Bansal and El-Sherif, 2004).

Field detection of organophosphate nerve agents currently relies on gas chromatography and mass spectroscopy (Diehl-Faxon et al., 1996). The primary equipment used for organophosphate detection in the DoD is the Hazardous Air Pollutants on Site (HAPSITE), a field portable gas chromatograph mass spectrometer (Goltz et al., 2011). The HAPSITE requires trained personnel, costs over a quarter of a million dollars, and requires complicated sample preparation and time-consuming analysis (Goltz et al., 2011). These deficiencies of the HAPSITE have motivated investigators to search for more useful detection technologies (Liu and Lin, 2006). Recently there have been considerable efforts to develop more practical sensors (Upadhyayula, 2011). Much work has focused on application of electrochemical
biosensors for chemical agent detection because they are relatively simple to make and can be easily tailored to suit specific requirements (Upadhyayula, 2011). Advancements in the field of nanotechnology and the coupling of nanotechnology and biosensors have led to development of nanotube-modified biosensors (Berger, 2008).

Organophosphate sensors can be designed to detect the organophosphate hydrolase enzyme (direct detection) or to detect cholinesterase inhibition (indirect detection) (Liu and Lin, 2006). Direct detection typically measures the pH change caused by production of acetic acid during hydrolysis of acetylcholine into choline and acetic acid (Mulchandani et al., 1999). Indirect detection uses an amperometer to measure the decrease in production of thiocholine, which is a product of acetylthiocholine (ASCh) hydrolysis, due to the presence of an OP (Liu and Lin, 2006). In vivo, ACh is hydrolysed by AChE. In vitro, ASCh is typically used in place of ACh because when hydrolysed by AChE, ASCh produces thiocholine, which, when oxidized by an electric current in an amperometer, can be easily detected using screen-printed electrodes (SPEs) (Alegret and Merkoci, 2007). In the presence of an OP, thiocholine production is decreased, resulting in a measurable current decrease.

Previous work done in this laboratory explored whether peptide nanotubes on Nafion-stabilized screen-printed electrodes could enhance the electrochemical signal of AChE when exposed to an organophosphate in solution (Stevens, 2012). In this study, we adapted these techniques for vapor phase OP detection. A jacketed voltammetry cell with a gold screen-printed electrode was used for gas phase detection in place of the carbon screen-printed electrode in an electrochemical solution that was used for liquid phase detection. The gold electrodes were used because they have been shown to have a
higher sensitivity than carbon electrodes, a fast response and good reproducibility (Xu et al., 2004). Also, based on Park (2010), we encapsulated HRP within the PNTs to boost our response signal strength. A third major difference in this study was that two electrode configurations were tested; one more suited to the laboratory and the other more suited to field application. The ultimate goal of this line of research is to create a small and affordable sensor that can detect organophosphate in the air in real-time and at concentrations below the lethal limit, which in the case of VX gas, the most potent organophosphate nerve agent, is a ten-minute exposure at 0.03 ppmv (Hulet et al., 2007) or 0.04 ppmv for sarin (Arduini et al., 2010). Such a device could be carried by a combatant in a theater of war, affixed to points on the perimeter of a base or camp, or attached to a small unmanned aerial vehicle (UAV) for forward reconnaissance. In addition to military applications, such a device would be affordable and easy to use so that it could be made commercially available to agricultural workers, domestic emergency services personnel, and other commercial applications where testing the air or water for potentially dangerous levels of pesticides or other OP products is called for.

The findings of this study are presented in a scholarly article in Chapter 3.

II. Literature Review

Organophosphates

Organophosphates are esters of phosphoric acid (Figure 1) (Stevens, 2012). Organophosphates are found extensively both in nature and as synthetic compounds. In nature, polyphosphates make up such important structures as DNA, RNA, and ATP.
Synthetically, organophosphates are used in a wide variety of products such as flame retardants, solvents and lubricants, and pesticides.

Toxic organophosphate compounds were investigated as pesticides during the 1920s and 1930s, but it was not until 1937 that their potential as chemical warfare agents was recognized (Marrs, 1993). In 1936 in Germany, the organophosphate Tabun was being examined for use as a pesticide by Dr. Gerhard Schrader. The accidental exposure of a laboratory assistant to Tabun vapor made it quite clear that the compound had potential military use as well, an observation that Dr. Schrader passed along to the German government. The following year, the German government took over the research and began producing Tabun and filling munitions with it, producing over 12,000 tons of the nerve agent over the course of WWII (Van der Leun, 1972).
Organophosphate nerve agents act by blocking the site on acetylcholinesterase (AChE) that would normally attach to acetylcholine (ACh) and catalyze its hydrolysis, producing acetic acid and choline (Figure 2 and Figure 3). ACh stimulates muscles, and when an OP agent has irreversibly bound to AChE, ACh accumulates, resulting in continuous stimulation of muscle groups. In particular, the diaphragm cannot relax, causing death by suffocation within minutes (Bansal and El-Sherif, 2004).

![Figure 2. Normal acetylcholine hydrolysis (ATSDR, 2010)](image1)

![Figure 3. Inhibition of AChE catalysis by OP (ATSDR, 2010)](image2)
Biosensors

A biosensor is a device that uses biological materials to detect the presence of chemicals. In this study we are using self-assembled peptide nanostructures, the enzyme acetylcholinesterase (AChE), and a substitution of its sympathetic protein, acetylcholine (ACh). Versions of biosensors were in use as early as 1922 (Tokas et al., 2000). One of the first and still most common uses of biosensors is glucose testing for diabetics (see Table 1). Medical testing has predominantly driven the development of biosensors and initially led to the use of biosensors in other medical equipment for the specific detection of proteins or enzymes (Tokas et al., 2000). Today biosensors are used in food analysis, bio-molecular research, drug development, crime detection, medical diagnosis, environmental monitoring, quality control, industrial process control, pharmaceutical manufacturing and biological warfare agent detection (Tokas et al., 2000). In 1995-1996, scientists began developing biosensors to detect organophosphorus pesticides (Diehl-Faxon et al., 1996; Imato and Ishibashi, 1995; Trojanowicz and Hitchman, 1996). In a recent study, Scognamiglio et al. (2012) developed an integrated biosensor for the detection of an array of endocrine-disrupting chemicals to include OPs in the liquid phase. Stevens (2012) specifically examined the use of a cholinesterase inhibiting biosensor for OP detection in liquid, using nanostructures to miniaturize the biosensor onto a 2mm diameter working electrode.
Table 1. Important events in the development of biosensors (Taylor and Schultz, 1996)

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td>First report on immobilization of proteins on activated charcoal</td>
</tr>
<tr>
<td>1922</td>
<td>First glass pH electrode (first biosensor)</td>
</tr>
<tr>
<td>1962</td>
<td>First time the term “biosensor” is used and defined: an amperometric enzyme electrode for glucose</td>
</tr>
<tr>
<td>1969</td>
<td>First potentiometric biosensor: urease immobilized on ammonia</td>
</tr>
<tr>
<td>1975</td>
<td>First description of a fiber optic sensor with immobilized indicator to measure CO2</td>
</tr>
<tr>
<td>1975</td>
<td>First commercial biosensor (glucose)</td>
</tr>
<tr>
<td>1980</td>
<td>First fiber optic pH sensor for blood gasses</td>
</tr>
<tr>
<td>1982</td>
<td>First fiber optic biosensor for glucose</td>
</tr>
<tr>
<td>1983</td>
<td>First plasmon resonance immunosensor</td>
</tr>
<tr>
<td>1984</td>
<td>First mediated amperometric biosensor (glucose)</td>
</tr>
<tr>
<td>1987</td>
<td>Blood-glucose biosensor made commercially available</td>
</tr>
<tr>
<td>1992</td>
<td>Hand-held blood-glucose biosensor made commercially available</td>
</tr>
<tr>
<td>1996</td>
<td>Glucose test strips made commercially available</td>
</tr>
</tbody>
</table>

**Cholinesterase biosensors**

Cholinesterase biosensors have been used since 1962 for the detection of heavy metals, cocaine, nicotine and other drugs, toxins, fluoride, glycoalkaloids, insecticides, and nerve agents (Arduini et al., 2010). Specifically for OP detection, there are several different models of biosensors. In the liquid phase, solid state and sol-gel fiber-optic photometers, spectrophotometric, chemiluminescent, Surface Plasmon Resonance, and piezoelectric biosensors have been used (Arduini et al., 2010). In the gas phase, tin oxide nanowires, network films of single-walled carbon nanotubes, and surface acoustic wave sensor arrays employing a variety of different polymers as sensing materials have been experimented with (Arduini et al., 2010). They have all been effectively used to detect OP nerve agents in the ppm range. However, none of these sensors are as simple to create or as sensitive to nerve agents as cholinesterase biosensors, which can reliably
detect nerve agents in the ppb range, both in liquid and gas-phase tests (Arduini et al., 2012). Cholinesterase biosensors are biosensors that are specifically designed to detect the interaction between the neurotransmitter acetylcholine and the hydrolyzing enzymes acetylcholinesterase (AChE) or butyrylcholinesterase (BChE). AChE biosensors detect indirectly by inhibition, hydrolyzing ASCh and producing thiocholine, which is subsequently oxidized by an electrode via a current that is produced and measured by a potentiometer. Introduction of an organophosphate into the system will reduce the amount of ASCh hydrolyzed, thus reducing the current required to oxidize the thiocholine. BChE is used in direct-detection methods, using BChE antibodies immobilized on a surface to collect BChE and measuring the enzyme activity (Du et al., 2011). Cholinesterase biosensors are simple and inexpensive and have a high sensitivity (Arduini et al., 2007; Sharma et al., 2012). Two prototype cholinesterase biosensors for gas-phase detection have been tested. Mlsna et al. (2006) were able to detect soman, sarin and tabun, though optimal detection was only achieved at 90°C. Arduini et al. (2012) were able to detect sarin and VX but not below the lethal concentrations. However, these tests represent a significant advance towards the production of an affordable mass-produced OP biosensor.

One of the drawbacks of cholinesterase biosensors is due to the fact that the reaction of the OP with the enzyme is irreversible. This is a limiting factor in a field-deployable biosensor, as it makes production of a reusable sensor problematic. However, recent studies have devised two possible methods for producing reusable electrodes. Di Tuoro et al. (2011), based on an enzyme reactivation method by Gulla et al. (2002), were able to apply an oxime to their electrode with 95% reactivation after one use and 60%
reactivation after eleven uses. Du et al. (2011) reported varying responses to reactivation, depending on the OP measured. In the case of aflotoxin detection using AChE, the reaction is naturally reversible in the absence of the aflotoxin (Arduini et al., 2007).

**Nanotubes**

Carbon nanotubes were discovered in 1991 (Iijima, 1991). Since then they have been extensively studied for their unique electrical properties that make them suited for amperometric and potentiometric studies in biosensors. Kong et al. (2000) built the first carbon nanotube biosensor for detecting the amine group (-NH₂) and ammonia gas. Chen et al. (2001) were able to immobilize proteins on carbon nanotubes, and in 2003 carbon nanotubes were used on biosensors to detect glucose (Yemini et al., 2005). In 2005, while searching for a self-assembled fragment of the β-amyloid polypeptide in conjunction with Alzheimer's research, peptide nanotubes (PNTs) were discovered (Yemini et al., 2005). PNTs are self-assembled, biocompatible and easily modified, and have been shown to greatly increase the sensitivity of biosensors (Yemini et al., 2005).

A number of methods and adaptations of nanostructures have been studied to increase the performance of biosensors. The use of nanostructures on the electrode serves to dramatically increase the surface area that the enzyme can attach to, thus increasing the sensitivity of the electrode (Park et al., 2011). The PNTs also serve to hold and protect the enzyme, immobilizing it on the electrode. This helps protect the enzyme from physical agitation; possibly being knocked or washed off the surface of the electrode during transport or use. Stevens (2012) demonstrated the use of PNTs on a cholinesterase biosensor to increase the response of SPEs when measuring the residual
activity of the enzyme AChE in the liquid phase in the presence of OP. Carbon SPEs were modified with PNTs and then coated with AChE. These modified SPEs showed an increase in amperometric response when compared to unmodified SPEs (Stevens, 2012). Additionally, an enzyme can be encapsulated within the hollow structure of the peptide nanotubes. Encapsulation further protects the enzyme from physical disturbance as well as natural degradation by limiting its exposure to the environment. This has been shown to increase the longevity of the electrode (Park et al., 2011). Encapsulation works well with the hollow structure of peptide nanotubes; as the thin walls of the nanotube minimize signal reduction, while immobilizing and protecting the enzyme, thereby increasing its stability and longevity. The limiting factor of enzyme encapsulation is that the interior surfaces of the nanotubes provide less surface area for attachment than the exterior surfaces. Encapsulating one enzyme within the peptide nanotubes and coating the nanotubes with another enzyme has proven effective at increasing both the stability and longevity of the biosensor (Park et al., 2011). Horseradish peroxidase (HRP) is used extensively in biochemistry for its ability to increase the signal of the target molecule (Carlsson et al., 2005). Encapsulating HRP within the peptide nanotubes and then coating the nanotubes with AChE or a similar enzyme has been shown to significantly increase the sensitivity of the electrode (Park et al., 2011). There are other techniques that have been applied separately or in conjunction with immobilization of enzymes on nanostructures to improve biosensor performance: entrapment and encapsulation of the target enzyme, encapsulation of a different enzyme, and cross-linking. Entrapment is a method of physically encasing the enzyme in a gel matrix or polymer. The advantage of this is that it firmly fixes the enzyme to the surface of the electrode. The drawback of
this method is that it creates a barrier between the enzyme and the substrate that
decreases the sensitivity of the electrode (Andreescu et al., 2002). Cross-linking is a
method of covalently binding the enzyme to the electrode. It creates a very strong bond
and provides good stability, but it causes some of the enzyme to denature in the process
and requires a significantly greater amount of enzyme (Andreescu and Marty, 2006).
Some combination of these methods can also be used for maximum effect.

In this study, detailed in the draft manuscript that follows, our goal is to adapt the
manufacture of the PNT-encapsulated biosensor used by Stevens (2012) to detect
dissolved OP for use as a gas phase detector. We accomplish this by using a gold
nanotube-modified SPE, treated with ASCh and AChE, to measure gas phase
concentrations of malathion.
III. Scholarly Article

Organophosphate vapor detection on gold electrodes using peptide nanotubes

Peter Baker\textsuperscript{a}, Mark Goltz\textsuperscript{a}, Amanda Schrand\textsuperscript{b}, Dong-Shik Kim\textsuperscript{c}

\textsuperscript{a} Department of Systems and Engineering Management, Air Force Institute of Technology, WPAFB, OH 45433

\textsuperscript{b} Integrated Sensing & Processing Sciences Branch, Munitions Directorate, Air Force Research Laboratory, Eglin AFB, FL 32542

\textsuperscript{c} Department of Chemical and Environmental Engineering, University of Toledo, Toledo, OH 43606

Abstract

Vapor-phase detection of the model organophosphate (OP) malathion was achieved using enzymes encapsulated in peptide nanotubes and attached to gold screen-printed electrodes. Malathion was chosen as the model for this experiment because its binding mechanism with acetylcholinesterase (AChE) is identical to its more potent counterparts (such as sarin or VX), but it is relatively nontoxic, which makes it both practical and easy to use. The peptide nanotubes had horseradish peroxidase encapsulated inside, to increase sensitivity, and were coated with both acetyltihiocholine (ASCh) and AChE on the outside. ASCh hydrolysis, which produces thiocholine, was catalyzed by the AChE. The thiocholine was then oxidized by the electrodes to produce a signal that could be measured by a cyclic voltammeter. This signal was inhibited in the presence of malathion vapor, with the extent of inhibition proportional to the malathion concentration down to the detection limit of the biosensor.

This study first established methods to consistently reproduce known concentrations of malathion vapor. Once the vapor concentration was established, peptide nanotube-modified gold screen printed electrodes were used to detect OP vapor. The nanotube-modified electrodes were first exposed to AChE, then to ASCh, and finally
inserted into an airtight vial with a known concentration of malathion. Cyclic voltammograms were taken at each step of the process to monitor the changes in activity. The biosensor response was inhibited by OP concentrations as low as 12 ppbv, which is less than half the lethal limit of 30 ppbv for VX.

This research demonstrates the sensitivity of nano-modified biosensors for the detection of organophosphate vapor, an important development in countering weaponized organophosphate nerve agents and detecting commercially-used pesticides.

Keywords: organophosphate vapor, peptide nanotubes, biosensor, acetylcholinesterase

1. Introduction

Since their inception in 1937, chemical warfare nerve agents such as sarin and VX have posed some of the deadliest threats in both conventional warfare and terrorist tactics (Upadhyayula, 2011). Current equipment for field detection of organophosphate nerve agents makes use of a “luggable” 70-pound GC/MS (Diehl-Faxon et al., 1996; Goltz et al., 2011). This equipment requires trained personnel, costs several hundred-thousand dollars, and requires complicated sample preparation and time-consuming analysis (Goltz et al., 2011). A feasible and far more practical alternative for field detection of OPs makes use of electrochemical biosensors, which are relatively simple to make and can be easily tailored to suit specific requirements (Upadhyayula, 2011; Arduini et al., 2012).

Organophosphate nerve agents irreversibly bind to AChE in the central nervous system, which prevents the nervous system from hydrolyzing its build-up of acetylcholine (ACh). ACh stimulates muscles, and when an OP agent has irreversibly bound to AChE, ACh accumulates, resulting in continuous stimulation of muscle groups.
In particular, the diaphragm cannot relax, causing death by suffocation within minutes (Bansal and El-Sherif, 2004). Organophosphate sensors can be designed to detect the organophosphate hydrolase enzyme (direct detection) or to detect cholinesterase inhibition (indirect detection). Direct detection typically uses a potentiometer to monitor the pH change caused by the production of acetic acid during hydrolysis of acetylcholine into choline and acetic acid (Mulchandani et al., 1999). Indirect detection uses an amperometer to monitor the decrease in concentration of thiocholine, which is a product of acetylthiocholine hydrolysis, due to the presence of an OP (Liu and Lin, 2006).

Previous studies have demonstrated various methods of using AChE-based biosensors to detect organophosphates in the liquid phase (Park et al., 2011; Stevens, 2012). While these liquid phase studies have important applications for pesticide detection in agricultural runoff and organophosphate concentrations in water used for chemical agent decontamination, limited research has been done to investigate application of AChE-based biosensors to detect organophosphate nerve agents in the atmosphere. Arduini et al. (2007) successfully developed and tested a Prussian blue-modified silver SPE using butyrylthiocholine (BTCh) and butyrylcholinesterase (BChE) in the presence of sarin gas. Using amperometric techniques similar to those used in the liquid-phase studies, they were able to detect sarin in the gas phase at concentrations as low as 12 ppbv in the laboratory, which is lower than the 40 ppbv sarin concentration that represents an immediate danger to life and health (Arduini et al., 2007). However, a field deployable prototype developed in 2012 was unable to achieve the sensitivity needed for a marketable OP biosensor (Arduini et al., 2012). The ultimate goal of this line of research is to create a small and affordable sensor that can detect organophosphate in the
air instantaneously and below the lethal limit of 30 ppbv for VX (Hulet et al., 2007) or 40 ppbv for sarin (Arduini et al., 2010). Such a device could be carried by a combatant in the field, affixed to points on the perimeter of a base or camp, or attached to a small unmanned aerial vehicle (UAV) for forward reconnaissance.

In this study, a gold SPE was used because it has been shown to have a higher sensitivity and better reproducibility than carbon SPEs and is cost-effective for mass production unlike glassy carbon electrodes (Xu et al., 2012).

Figure 4. Electrode Construction

Figure 4 shows the construction of the electrode used in this study. The electrode was modified with peptide nanotubes to increase the surface area for enzyme attachment, and which have been shown to increase the response in potentiometric detection (Stevens, 2012). HRP was encapsulated within the hollow structure of the PNTs. This
has been shown by Park et.al (2010) to increase the signal in potentiometric detection, thus decreasing the volume of enzyme required and the response time of the redox reaction. Encapsulating the HRP rather than introducing free HRP into solution protects the enzyme, thereby increasing the enzyme activity and longevity (Park et al., 2010). Nafion, used as a protective layer over the AChE-treated PNT-modified electrode, was shown to assist in immobilizing the enzyme on the PNTs, especially when in solution (Stevens, 2012). Similar to the in vivo reaction between acetylcholine and acetylcholinesterase, this study used acetylthiocholine (ASCh) instead of acetylcholine. The acetylcholinesterase breaks down the acetylthiocholine by the same mechanism to produce acetic acid and thiocholine (Badea et al., 2006). The thiocholine is then oxidized by the working electrode to produce dithiocholine. This transfer of electrons is detected and measured by a potentiostat.

2. Materials and Methods

2.1 Materials

AChE-type V-S from electric eel, 500 U/mg; horseradish peroxidase; and malathion >95% were purchased from Sigma Aldrich (Milwaukee, WI) and stored at -10°C. ASCh and H-Phe-Phe-OH were purchased from Sigma Aldrich (St. Louis, MO), and stored at 4°C. 1,1,1,3,3,3-hexafluoro-2-propanol (99.8% purity) was purchased from Sigma Aldrich (Milwaukee, WI) and stored at 4°C. Nafion© 117 solution (approx. 5%) was purchased from Sigma Aldrich (Allentown, PA). Deionized water was generated in the lab via reverse osmosis.
Gold screen-printed electrodes (model DRP-250) with a 4-mm-diameter gold working electrode and the electrode-potentiostat interface cable were purchased from Metrohm USA (Riverview, FL). The jacketed compact voltammetry cell was purchased from Pine Research Instrumentation (Durham, NC).

2.2 Apparatus

All electrochemical measurements were conducted using a Parstat 2273 Advanced Electrochemical System and PowerSuite ©Software from Princeton Applied Research. Media were dried using a Fisher Scientific Isotemp Vacuum Oven, model 280A, and centrifuged using an Eppendorf Centrifuge 5810R. Peptide nanotubes (PNTs) were agitated using a Cole-Parmer 8890 Sonicator. Experiments were conducted in a Pine Research Instrumentation jacketed compact voltammetry cell. The temperature within the cell was adjusted and maintained using a Cole-Parmer Polystat Temperature Controller. Malathion concentration was determined using an Agilent Technologies 6890N Network GC Systems model gas chromatograph mass spectrometer (GC/MS).

2.3 Methods

PNTs were synthesized by dissolving 100 mg of H-Phe-Phe-OH in 1 ml of 1,1,1,3,3,3-hexafluoro-2-propanol. This mixture was swirled gently by hand for a few seconds and then placed in a sonicator for five minutes to ensure complete dissolution.

To encapsulate the horseradish peroxidase (HRP) inside the PNTs, 1 ml of the PNT solution was dried overnight in a vacuum oven. To this was added 1 ml of 50 mM phosphate buffer solution containing 1 mg HRP. This mixture was vortexed briefly and then incubated in a shaker at 5°C, 120 rpm for one week. This allowed the HRP to lodge inside the hollow structure of the PNT via capillary action (Park et al., 2010). The
mixture was then centrifuged at 4,000 rpm for 1 hour. The liquid was withdrawn with a
micropipette, and the PNT mixture was washed with 4 ml of 5.8M phosphate buffer
solution and then centrifuged again for 1 hour. This process was repeated twice more.
After the liquid was withdrawn the third time, 2 ml of 5.8M phosphate buffer solution
was added, and the PNTs containing the encapsulated HRP were refrigerated. This
process was confirmed to have encapsulated the HRP via scanning transmitting electron
microscopy (STEM) analysis (Park et al., 2010).

Malathion vapor concentration was determined by comparing peak areas on a
GC/MS of known liquid concentrations of malathion to peak areas of unknown vapor
concentrations. A calibration curve was established using 10 points of known-
concentration malathion standards from 0.1 ppm to 100 ppm. Malathion vapor was
obtained by pipetting 2 mL of 95% pure malathion into a 40-ml amber vial with a gas-
tight septum cap. Malathion (vapor pressure $4 \times 10^{-5}$ mm Hg at 30%) was allowed to
equilibrage with the atmosphere inside the vial until saturation. A locking gas-tight
syringe was then used to transfer 2 mL of the gas inside the vial into the GC/MS via a
direct injection port.

Vapor concentration was adjusted by injecting a known volume of gas saturated
with malathion at its vapor pressure (known concentration) into a vial of known volume
purged with nitrogen at a constant temperature. The resulting concentration can then be
calculated after equilibration is achieved.

The sensors were prepared by first depositing 5µL of PNTs containing the
encapsulated HRP on the working electrode, which was then allowed to dry in a hood at
room temperature and pressure (average of 65°C and 745 mm Hg). Then, 5µL of
1000U/mL AChE was deposited on top of the PNTs and allowed to dry. Over that, 5µL of Nafion® was deposited and allowed to dry. The electrode was then placed into a voltammetry flask filled with phosphate buffer solution, and cyclic voltammetry measurements were taken. The electrodes were then removed from the phosphate buffer solution and inserted into an identical vial with phosphate buffer solution containing 1 mmol ASCh, and cyclic voltammetry measurements were taken again. The electrodes were then immediately transferred into a vial purged with nitrogen or into a vial containing 25 ppbv malathion vapor, and cyclic voltammetry measurements were taken again.

A longevity experiment was conducted at fifteen, thirty, forty-five, and sixty days after electrode construction. The electrodes were tested with two different preparation methods and for each preparation method, two different tests were applied for a total of four different tests (see Table 2). These different methods were investigated to help determine what options might be available for preparation and use of viable field-deployable sensor. For the longevity experiments, two sets of electrodes were initially prepared. One set was prepared with just PNTs and one set had PNTs covered with AChE (Table 2). Considering the ultimate goal of developing a field-deployable biosensor, it would be beneficial if the electrode could be prepared with AChE, so in the field it would only be necessary to add ASCh to activate the biosensor. The electrodes were stored dry at 4°C. At the aforementioned time intervals, the electrodes were tested using cyclic voltammetry measurements.
Table 2. Longevity experiment setup

<table>
<thead>
<tr>
<th></th>
<th>Preparation 1</th>
<th>Preparation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Electrodes prepared with PNTs and AChE</td>
<td>Electrodes prepared with just PNTs</td>
</tr>
<tr>
<td>Treatment 1 (Wet)</td>
<td>Treated with Nafion and immersed in phosphate buffer solution containing ASCh</td>
<td>Treated with AChE and Nafion and immersed in phosphate buffer solution containing ASCh</td>
</tr>
<tr>
<td>Treatment 2 (Dry)</td>
<td>Treated with 5 µL ASCh</td>
<td>Treated with 5 µL AChE and 5 µL ASCh</td>
</tr>
<tr>
<td>Residual activity test</td>
<td>Inserted into 25 ppbv malathion gas</td>
<td>Inserted into 25 ppbv malathion gas</td>
</tr>
</tbody>
</table>

*Prepared With PNTs and AChE And Tested Wet*

One set of electrodes was prepared with 5 µL of PNTs encapsulating HRP. This was allowed to dry and then 5 µL of AChE was deposited on the PNTs and allowed to dry. The electrodes were then stored for the longevity tests. Just prior to testing, 5 µL of Nafion was added on top of the AChE and allowed to dry. Once dry, the electrode was immersed in phosphate buffer solution and a cyclic voltammogram was taken. Then the electrode was removed from the phosphate buffer solution and immersed in a phosphate buffer solution with 1 mmol ASCh and a cyclic voltammogram was again taken. Finally, the electrode was inserted into a gaseous environment containing 25 ppbv malathion gas and another cyclic voltammogram was taken.

*Prepared With PNTs and AChE And Tested Dry*

One set of electrodes was prepared with 5 µL of PNTs encapsulating HRP. This was allowed to dry and then 5 µL of AChE was deposited on the PNTs and allowed to dry. The electrodes were then stored for the longevity tests. Just prior to testing, 5 µL of ASCh was added on top of the AChE and the electrode was immediately inserted into a nitrogen gas environment and a cyclic voltammogram was taken. Then the electrode was
removed from the nitrogen gas and placed in a gaseous environment containing 25 ppbv malathion gas and another cyclic voltammogram was taken.

**Prepared With PNTs Only And Tested Wet**

One set of electrodes was prepared with 5 µL of PNTs encapsulating HRP. The electrodes were then stored for the longevity tests. Just prior to testing, 5 µL of AChE and 5 µL of Nafion was added on top of the PNTs and after each addition, allowed to dry. Once dry, the electrode was immersed in phosphate buffer solution and a cyclic voltammogram was taken. Then the electrode was removed from the phosphate buffer solution and immersed in a phosphate buffer solution with 1 mmol ASCh and a cyclic voltammogram was again taken. Finally, the electrode was inserted into a gaseous environment containing 25 ppbv malathion gas and another cyclic voltammogram was taken.

**Prepared With PNTs Only And Tested Dry**

One set of electrodes was prepared with 5 µL of PNTs encapsulating HRP. The electrodes were then stored for the longevity tests. Just prior to testing, 5 µL of AChE and 5 µL of ASCh were added on top of the PNTs and the electrode was immediately inserted into a nitrogen gas environment and a cyclic voltammogram was taken. Then the electrode was removed from the nitrogen gas and placed in a gaseous environment containing 25 ppbv malathion gas and another cyclic voltammogram was taken.

A response vs. concentration test was conducted by injecting a known volume of malathion-saturated gas into a known volume of nitrogen. For this test, a 50-mL gastight syringe was used. Varying mixtures of the 25 ppbv malathion gas and nitrogen gas were drawn into the syringe for a total of 50 mL, which was then allowed to sit at 20°C
for an hour to allow the gases to mix evenly. The entire volume was then injected through a septum into the 25-mL voltammetry flask containing the electrode. The mixture was tested at 0, 10, 25, 50, 70, 75, 80, 90, and 100% of the initial 25 ppbv malathion vapor. At each concentration, each electrode was tested first in phosphate buffer, then phosphate buffer with ASCh, and finally in the malathion-nitrogen vapor mix to establish inhibition of the reaction in terms of percent change. At each phase, a cyclic voltammogram was generated. Replicate experiments for each concentration were averaged.

3. Results and Discussion

The cyclic voltammogram in Figure 5 establishes a baseline oxidation peak for the reaction between AChE and ASCh. The potentiostat measures the oxidation of thiocholine, which is a product of the hydrolysis of the enzymatic reaction with AChE and ASCh. The bottom (thin) line is the response when there is no ASCh present, and therefore there is no reaction (1.6 X 10^{-6} A). Note that this line does not overlap zero on the x-axis of the grid. This minimal response is presumed to be “noise” from the potentiostat. This measured value for the buffer solution without any ASCh (and therefore presumably no reaction), is used as “zero” in all subsequent calculations. The top (thick) lines represent the response in the presence of the reaction (4.8 X 10^{-4} A).
The next step of the experiment was to determine if malathion vapor could be observed to inhibit the enzymatic reaction of AChE and ASCh. The thin film of phosphate buffer containing ASCh that remained on the electrode after it was removed from solution continued the reaction. Ideally, there would be no decrease in reaction when the sensor is placed in a nitrogen atmosphere. The decrease shown in Figure 6a is assumed to be due to less available ASCh after the electrode is removed from solution. Comparable biosensor tests in the literature do not require removal of the enzyme-coated electrode from the reactive substrate-containing media before introducing the OP (Arduini et al., 2007). This is preferred to the method we used, as it eliminates a potentially confounding factor (inhibition of the reaction due to removal of the electrode from the substrate-containing environment). Our method was used because of equipment...
limitations, as well as a desire to follow, as closely as possible, the earlier liquid phase studies conducted by Stevens (2012). That being said, when placed in the 25 ppbv malathion vapor, the response of our electrode dropped over 99% to near zero (Figure 6b). We can conclude from this that it is in fact the malathion vapor that inhibits the reaction between the enzyme and the substrate.
Figure 6. Current inhibition in either nitrogen gas (6a) or 25 ppbv malathion vapor (6b)
The longevity test indicated that the time elapsed was a much less significant factor than the method used. Those electrodes that were immersed in blank buffer solution and then buffer solution containing ASCh and placed in a nitrogen atmosphere showed an *increase* in sensitivity (response) over time (red and purple lines in Figure 7). Those treated with a 5-µl drop of ASCh and AChE and placed in a nitrogen atmosphere almost uniformly showed no response at all after 15 days, even though in both methods the AChE used on the electrodes prepared with PNTs only (red and blue lines in Figure 7) was fresh instead of some number of weeks old, depending on the trial.

![Figure 7. Results of the Longevity Test](image)

The longevity test was ultimately to test the efficacy of the electrode as an OP detector over time. During this experiment, the electrodes were spot tested in 25 ppbv malathion gas. Those electrodes that maintained AChE-ASCh reactivity showed a response that was uniformly consistent with the 25 ppbv tests done initially (prior to any elapsed time). Those electrodes that no longer had any AChE-ASCh reactivity showed
no response. Therefore, testing the reactivity of the enzyme over time was the key factor in determining the longevity of the electrode. Our results were comparable to Arduini et al. (2012) although their test was over a much longer duration. At times greater than 60 days, Arduini et al. (2012) did show some decrease in enzyme reactivity.

The final aspect of this study was to establish a response versus concentration curve for the biosensor. Results are shown in Figures 8 and 9. Based on Figure 8, it was determined that the inhibition response at concentrations below 12.5 ppbv was not related to the concentration, so a detection limit of 12.5 ppbv was established. Figure 9 indicates a linear response for malathion concentrations between 12.5 and 25 ppbv. This is encouraging, as this range of concentrations is below the lethal concentration for all known OPs.

Figure 8. Biosensor response vs. concentration (error bars indicate standard deviation of replicates)
Figure 9. Biosensor response vs. concentration (error bars indicate standard deviation of replicates) above detection limit

4. Conclusions

Under laboratory conditions, peptide-nanotube-modified biosensors appear to be a viable method of detecting organophosphate vapors at low concentrations. The detection limit for malathion found in this study is well below the lethal limit for all known OPs. The response time is nearly instantaneous. The ease and low cost of their construction, their extreme sensitivity, and their rapid response give peptide-nanotube-modified biosensors significant advantages over current detection methods. However, the conditions under which these tests were run could not be duplicated in the field. The reagents have to be refrigerated, the electrode requires full immersion in the ASCh solution, and the electrodes would require preparation by a trained laboratory technician within hours of their use. Additionally, the packaging and warning system of this proof of concept electrode would require further development before the biosensor would be a field-useable product. These preliminary results are encouraging, though, and further
research and development of this biosensor as a field-deployable OP biosensor should be pursued.

5. References:

IV. Conclusion

Additional Results and Discussion

This study has demonstrated that peptide nanotube-modified biosensors are viable devices for detecting organophosphate vapors; however, the conditions under which they can be used to detect organophosphate vapors restrict them to laboratory conditions at this time.

The longevity test showed some interesting and somewhat surprising results. What was surprising was that, for the timeframe we used, the “wet” biosensor configuration exhibited no decrease at all in enzyme response and in fact there was increased reactivity over the course of the study. This suggests that some combination of the peptide nanotubes and the Nafion serves to protect the enzyme from external conditions. Unfortunately, in the "dry" configuration, the enzyme appeared to lose activity quickly. This is unfortunate because the dry configuration lends itself better to field application, while the wet configuration, which requires submersion of the electrode into a substrate-containing solution, is more difficult to apply in the field. We suspect that the cause of this difference is that the Nafion forms a barrier that requires sufficient rehydration to bridge between the reactants and the electrode. So while ASCh and AChE may be reacting to produce thiocholine, with the dry configuration the thiocholine is not in electrical contact with the electrode, so there is no oxidation reaction to be detected by the potentiometer. Immersing the electrode into solution provides sufficient hydration to complete the circuit. Arduini et al. (2012) also postulated that Nafion might selectively
block thiocholine, thus limiting thiocholine oxidation. They suggest using cellulose acetate instead of Nafion.

Only five papers in the last 10 years have actually tested sensors for weaponized nerve agents (Lee et al., 2000; White and Harmon, 2005; Arduini et al., 2007; Pohanka et al., 2009; Upadhyay et al., 2009). They have all reported a significant difference in the sensitivity of detection (see table 3).

Table 3. Tests of weaponized nerve agents

<table>
<thead>
<tr>
<th>Method of Testing</th>
<th>Enzyme Used</th>
<th>Nerve Agent Used</th>
<th>Detection Limit</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amperometric modified SPE</td>
<td>BChE</td>
<td>Sarin VX</td>
<td>12 ppb 14 ppb</td>
<td>Arduini et al. (2007)</td>
</tr>
<tr>
<td>Potentiometric</td>
<td>AChE</td>
<td>Soman Sarin</td>
<td>0.018 ppb 0.084 ppb</td>
<td>Lee et al. (2000)</td>
</tr>
<tr>
<td>Amperometric SPE</td>
<td>AChE</td>
<td>Tabun Sarin</td>
<td>1.48 E-8 M 5.88 E-10 M</td>
<td>Pohanka et al. (2009)</td>
</tr>
<tr>
<td>Amperometric Modified Glassy Carbon</td>
<td>AChE-ChOx</td>
<td>Soman Cyclosarin</td>
<td>1.07 E-8 M 9.12 E-9 M</td>
<td>Upadhyay et al. (2009)</td>
</tr>
<tr>
<td>Planar Wave Guide Absorbance Spectroscopy</td>
<td>AChE</td>
<td>Sarin</td>
<td>0.1 ppb</td>
<td>White and Harmon (2005)</td>
</tr>
</tbody>
</table>

Malathion has the lowest human toxicity of any of the OP pesticides that are used as proxy OPs for testing. Also, of the 215 studies of OP biosensors reviewed by Arduini et al. (2010), only approximately 10% had been tested or used in real-world applications and none of those in the gas phase. There is a dearth of relevant research data for gas-phase detection.

More sophisticated testing systems are able to introduce OP directly into the system without removing or disturbing the electrode or interrupting the potentiometer. This eliminates the need to calculate the effects of removing the electrode from solution.
into an N$_2$-saturated environment or a malathion saturated environment. Unfortunately, our laboratory lacks the equipment to set up a closed-environment experiment that would allow constant monitoring and adjusting of the gas flows into the system and constant monitoring of the potential at the working electrode.

**Further Research**

The ultimate goal of this line of research is to produce a working, competitive sensor that can be deployed in a combat scenario. While we have proven that these nanotube peptide-modified biosensors can detect OP in the gas phase, they still cannot be used in practice. The enzymes have to be stored at -20°C, the substrate at 4°C. However, glucose oxidase that is used for blood glucose monitoring devises and peroxidase enzymes such as horseradish peroxidase that are used for AIDS virus detection kits (ELISA) are known to last more than a year (Arduini et al., 2010). Sigma-Aldrich, the manufacturing company of the AChE we used, claims that AChE can maintain its activity for over a year if properly stored (at -20°C). Because the sensor has to be packed and stored in a dry state until being unpacked and used for sensing, the enzyme has to sustain its activity under dry conditions at an ambient temperature for a long time. It may be possible that if the enzyme is freeze dried (lyophilized), it can last at least a year. Then, when it needs be used, the sensor could be reanimated by being dipped in an electrolyte solution, and then contacted with the gas phase sample. It may also increase the life of the enzyme if the enzyme is encapsulated inside PNTs similarly to how we encapsulated the HRP in this study. Arduini et al. (2010) claims that a longer incubation time (contact time between enzyme and inhibitor) increases inhibition. The limit of this should be
explored to determine an optimal and realistically feasible incubation time for a forward-deployable biosensor; a compromise between sensitivity of the electrode and potential injury to the person holding the sensor. Literature has indicated incubation times from five minutes to two days may improve detection (Arduini et al., 2010). These possibilities bear investigation and may further the goal of developing a field-deployable biosensor.

**Bibliography**


# Development of Peptide Nanotube-Modified Biosensors for Gas-Phase Organophosphate Detection

## Abstract
Vapor-phase detection of the model organophosphate malathion was achieved using enzymes encapsulated in peptide nanotubes and attached to gold screen-printed electrodes. Malathion was chosen as the model for this experiment because its binding mechanism with acetylcholinesterase (AChE) is identical to its more potent counterparts (such as sarin or VX), but it has an extremely low human toxicity, which makes it both practical and easy to use. The peptide nanotubes had horseradish peroxidase encapsulated inside and were coated with both acetylthiocholine (ASCh) and AChE on the outside. ASCh hydrolysis, which produces thiocholine, was catalyzed by the AChE. The thiocholine was then oxidized by the electrodes to produce a signal that could be measured by a cyclic voltammeter. This signal was inhibited in the presence of malathion vapor, with the extent of inhibition proportional to the malathion concentration.

A calibration curve was first established in order to determine the concentration of malathion vapor in a given environment using standards of known concentrations of liquid malathion in a gas chromatograph. Once the vapor concentration was established, peptide-nanotube-modified, gold screen-printed electrodes were used to detect organophosphate vapor. The nanotube-modified electrodes were exposed to both AChE and ASCh and inserted into an airtight vial with a known concentration of malathion. Cyclic voltammograms were taken at each step to monitor the changes in activity.

This research demonstrates the ability to use nano-modified biosensors for the detection of organophosphate vapor, an important development in countering weaponized organophosphate nerve agents and detecting commercially-used pesticides.

## Subject Terms
Organophosphate, peptide nanotube, acetylcholinesterase