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IMPROVING THE STABILITY OF AN OXIME BASED ELECTROCHEMICAL
MICROSENSOR FOR ORGANO-PHOSPHATE VAPOR DETECTION

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Chemical Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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Abstract

A micro gas sensor has been developed by our group for the detection of organophosphate vapors using an aqueous oxime solution. The analyte diffuses from the high flow rate gas stream through a porous membrane to the low flow rate aqueous phase. It reacts with the oxime PBO (1-Phenyl-1,2,3,-butanetrione 2-oxime) to produce cyanide ions, which are then detected electrochemically from the change in solution potential.

Previous work on this oxime based electrochemistry indicated that the optimal buffer pH for the aqueous solution was approximately 10. A basic environment is needed for the oxime anion to form and the detection reaction to take place. At this specific pH, the potential response of the sensor to an analyte (such as acetic anhydride) is maximized. However, sensor response slowly decreases as the aqueous oxime solution ages, by as much as 80% in first 24 hours.

The decrease in sensor response is due to cyanide which is produced during the oxime degradation process, as evidenced by the cyanide selective electrode. Solid phase microextraction carried out on the oxime solution found several other possible degradation products, including acetic acid, N-hydroxy benzamide, benzoic acid, benzoyl cyanide, 1-Phenyl 1,3-butadione, 2-Isonitrosoacetophenone and an imine derived from the oxime. It was concluded that degradation occurred through nucleophilic attack by a hydroxide or oxime anion to produce cyanide, as well as a nitrogen atom rearrangement similar to Beckmann rearrangement.

The stability of the oxime in organic solvents is most likely due to the lack of water, and specifically hydroxide ions. The reaction between oxime and organo-phosphate to produce cyanide ions requires hydroxide ions, and therefore pure organic solvents are not compatible with the current micro-sensor electrochemistry. By combining a concentrated organic oxime solution with the basic aqueous buffer just prior to being used in the detection process, oxime degradation can be avoided while preserving the original electrochemical detection scheme.

Based on beaker cell experiments with selective cyanide sensitive electrodes, ethanol was chosen as the best organic solvent due to its stabilizing effect on the oxime, minimal interference with the aqueous electrochemistry, and compatibility with the current microsensor material (PMMA). Further studies showed that ethanol had a small effect on micro-sensor performance by reducing the rate of cyanide production and decreasing the overall response time.
To avoid incomplete mixing of the aqueous and organic solutions, they were pre-mixed externally at a 10:1 ratio, respectively. To adapt the microsensor design to allow for mixing to take place within the device, a small serpentine channel component was fabricated with the same dimensions and material as the original sensor. This allowed for seamless integration of the microsensor with the serpentine mixing channel.

Mixing in the serpentine microchannel takes place via diffusion. Both detector potential response and diffusional mixing improve with increased liquid residence time, and thus decreased liquid flowrate. Micromixer performance was studies at a 10:1 aqueous buffer to organic solution flow rate ratio, for a total rate of 5.5 µL/min. It was found that the sensor response utilizing the integrated micromixer was nearly identical to the response when the solutions were premixed and fed at the same rate.
Dedicated to my family and friends
Acknowledgments

I would like to thank my family and friends for all their love and support throughout my many years of education.

I would like to thank Ilwhan Oh for mentoring me when I first joined the research group and Chelsea Monty for being a great collaborator on many of the micro-sensor projects. Thanks also go to all my co-workers for the invaluable advice and moral support. Finally, I would like to acknowledge all the support from my advisor Dr. Richard Masel, whose ideas and guidance allowed me to succeed academically.

This work is supported in part by the Defense Advanced Research Projects Agency under U.S. Air Force grant FA8650-04-1-7121. Any opinions, findings and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the Defense Advanced Research Projects Agency or the U.S. Air Force.
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\(C_{if}\) concentration of species \(i\) in the fiber coating, M
\(C_{is}\) concentration of species \(i\) in the aqueous sample, M
\(C_{io}\) concentration of species \(i\) in aqueous solution initially, M
\([\text{CN}^-]\) concentration of cyanide ions in solution, M
\(\Delta E\) magnitude of potential change of solution, mV
\(K_i\) distribution coefficient of species \(i\)
\(L\) characteristic length of fluid flow geometry,
\(n_i\) total amount of species \(i\) in fiber, mol
\(pK_a\) logarithm of acidity constant
\(v\) velocity of fluid
\(V_f\) volume of fiber coating, L
\(V_s\) volume of aqueous sample, L
\(t\) time for complete diffusive mixing, s
\(L_{\text{mixing}}\) length over which the mixing occurs, \(\mu m\)
\(D\) diffusivity of one fluid in the other, \(cm^2/s\)
Greek Symbols

\[ \rho \quad \text{density of fluid} \]
\[ \mu \quad \text{viscosity of fluid} \]
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CN ISE</td>
<td>cyanide ion selective electrode</td>
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<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<tr>
<td>DVB</td>
<td>divinylbenzene</td>
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<tr>
<td>FID</td>
<td>flame ionization detection</td>
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<td>FTIR</td>
<td>fourier transform infrared spectroscopy</td>
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<td>GC</td>
<td>gas chromatograph</td>
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<td>MS</td>
<td>mass spectrometer</td>
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<td>OP</td>
<td>organophosphate</td>
</tr>
<tr>
<td>PA</td>
<td>polyacrylate</td>
</tr>
<tr>
<td>PAO</td>
<td><em>anti</em>-pyruvic aldehyde 1-oxime</td>
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<td>PBO</td>
<td>1-phenyl-1,2,3,-butanetione 2-oxime</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>ppb</td>
<td>parts per billion</td>
</tr>
<tr>
<td>Re</td>
<td>Reynolds number</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>reverse phase high performance liquid chromatography</td>
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<tr>
<td>SAW</td>
<td>surface acoustic wave</td>
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<td>SPME</td>
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Chapter 1: 
Introduction

In this age of information, nearly instantaneous results, and ultra portable technology, the need for micro-scale sensors has never been greater. One of the most important fields of development in sensor technology is for the detection of dangerous or deadly air borne chemicals. This includes compounds such as toxic organophosphates, explosives, industrial waste, and other naturally occurring harmful compounds. Although there are a wide variety of sensors, each tailored to a specific type of compound, the general requirements of any sensor detecting harmful chemicals are the following:

- High sensitivity
- High selectivity
- Portable
- Rapid
- Vapor phase detection ability

Such a sensor must be sensitive enough to detect the target compound(s) in sub-part per billion (ppb) concentrations, long before the exposure level becomes fatal. Due to the critical purpose of the sensor, it must demonstrate a high selectivity towards its intended target compound(s). The rate of false positives, as well as false negative results should be very low, as to avoid any inaccuracies. These sensors have to be portable, and able to be operated by a single person. Sample gathering, processing and analysis must all be carried out in the same portable unit. Additionally, the time scale for total operation ideally would take minutes, if not seconds to complete. Finally, such sensors must be able to detect its target chemical in vapor form, so that the operator may avoid contact with the liquid or solid source of the hazardous chemical.

Detection methods can be easily split according to their selectivity toward their target compound. Non-selective detection methods such as photovoltaic sensors, surface acoustic wave (SAW) sensors, micro-cantilever beam arrays, differential mobility spectrometers, carbon nanotube resistors, solid oxide based sensors, and other chemiresistive sensors can all be paired with a gas chromatography column to provide a measure of selectivity to the system.
based on column elution times. However, this adds to the total detection time of the sensor and if any compounds co-elute, the sensor will not be able to resolve the identities of all compounds. Additionally, most of the methods outlined above do not have the sensitivity to reach the ppb detection levels needed for effective operation. Highly selective detection methods include most biologically based methods, such as enzymatic sensors, as well as fluorescent chemosensors, and metal chelate based catalysts. Bio-sensors are often slower than non-selective sensors, and in the case of enzymatic sensors, lack long term stability and are single use only.

Of particular importance for detecting are acetylcholinesterase inhibitors, specifically toxic organophosphates (OP). Acetylcholine is a neurotransmitter produced at chemical synapses and is commonly found in neuromuscular and interneuronal transmission. Electrical signals are passed between cells through the synaptic cleft when acetylcholine is released by the axon terminal of the transmitting cell. Acetylcholine then diffuses towards the dendritic spine, where it stimulates the receptors on the receiving cell before being hydrolyzed by acetylcholinesterase. Figure 1.1 depicts the acetylcholine neurotransmission process.

![Figure 1.1: Operation of a cholinergic synapse. Acetylcholine is released, triggers receptors, is hydrolyzed to choline and acetate, and then is re-adsorbed.](image-url)
It is this swift destruction of acetylcholine that allows for rapid intercellular communication. Acetylcholinesterase inhibitors derive their toxicity from their ability to bind to the enzymes’ active site, thus preventing acetylcholine from being hydrolyzed. This in turn causes a buildup of acetylcholine in the synapse, which over stimulates receptors, and could eventually lead to a major disruption in normal nervous system activity.

Acetylcholinesterase inhibition by organophosphates is usually irreversible under normal conditions; however such poisoning can be reversed by introduction of a number of compounds based on the oxime functional group ($R_1R_2C\equiv\text{NOH}$)\textsuperscript{33}. The oxime functional group can easily lose a proton in aqueous solutions to become an oxime anion; a strongly nucleophilic species with a high affinity towards toxic (reactive) organophosphates, as well as other acid anhydride compounds. The phosphonyl-oxime intermediate which is formed quickly decomposed to yield the hydrated organophosphate, and fragments of the original oxime (figure 1.2)\textsuperscript{34,35}.

![Reaction Mechanism]

**Figure 1.2:** General reaction mechanism for oxime reacting with an organophosphate to form the unstable intermediate, which quickly breaks down to yield cyanide.
In the process of this reaction, the organophosphate unbinds with the enzyme, allowing it to continue functioning as normal. Many therapeutic oxime compounds such as Pralidoxime, Obidoxime, Methoxime, HI-6 and HLö-7 have been developed for the treatment of organophosphate poisoning based on this reaction mechanism\textsuperscript{36}.

This selective oxime based chemistry can be utilized for the detection of toxic organophosphates. The cyanide produced can be measured spectrophotometrically via the König color change reaction\textsuperscript{37} or through an electrochemical detection scheme\textsuperscript{38}. By using 1-phenyl-1,2,3-butanetrione 2-oxime (PBO) and optimizing solution pH, Dr. Ilwhan Oh from our research group was able to maximize the amount of cyanide produced and showed that this electrochemical method could easily outperform other organophosphate detection methods. Transitioning from beaker scale to microscale sensing using this optimized method further decreased detection time while increasing sensitivity\textsuperscript{39}. The main drawback with the current iteration of this sensing technique lies in the long term instability of the oxime solution. The purpose of this project will be to develop an alternative way to store oxime in a liquid form which minimized degradation, without negatively affecting the electrochemical detection method, and without significantly changing the current microfluidic sensor design.
Chapter 2: Literature Review

The following chapter presents a general overview of the current literature relating to oxime compounds, possible degradation mechanisms and products, current solid phase micro-extraction (SPME) techniques, cyanide detection via potentiometric measurements, and fluid mixing designs on the microscale. This will provide a good background for the results that are discussed in chapter 5.

2.1 Oxime reaction mechanisms

As previously mentioned, the oxime chosen for use in this electrochemical detection scheme is 1-phenyl-1,2,3-butanetrione 2-oxime (PBO). This was based on the magnitude as well as the duration of the solution potential change in response to the addition of the analyte (acetic anhydride). Keto-oximes were the only type of oximes tested due to their activity in aqueous solutions. The carbonyl group(s) present in keto-oximes provide a point for nucleophilic attack and ensures that the compound cleaves between the carbonyl carbon and the carbon attached to the oxime group. Cleavage of the compound in this matter produces a cyanide ion as an end result. Of the oximes tested, the two diketo-oximes performed better than the monoketo-oximes. The diketo-oximes provide a larger conjugated structure with which to distribute negative charges in, leading to a more stable anion. This can be seen by comparing the $K_a$ values of mono and diketo-oximes. The $K_a$ for PBO is more than an order of magnitude lower than that of a comparable mono-keto oxime such as anti-pyruvic aldehyde 1-oxime (PAO), indicating the anion PBO has a lower affinity for protons than PAO. It can also be seen that when comparing acyl groups in keto oximes, the benzoyl group results in a lower oxime reaction rate compared to that of acetyl. Although the exact reason for this is unknown, it may be due to ease of acyl cleavage from the oxime molecule as well as steric effects.
Ford and Watts performed a detailed investigation of the reaction mechanisms of PBO with the organophosphate isopropyl methylphosphonofluoridate. They found that in addition to the type of reaction shown in figure 1.2, a secondary reaction was also taking place. The overall mechanism given in their paper is shown in figure 2.1.

![Reaction Mechanism Diagram]

**Figure 2.1:** Expanded reaction mechanism of PBO with a reactive organophosphate.

This secondary mechanism was proposed in order to account for the larger than 1:1 ratio of oxime to organophosphate consumed in the reaction at pH values less than ~8. In this secondary reaction, the oxime-phosphate intermediate complex is attacked not by a hydroxide ion but instead by another oxime anion. This attack takes place on the carbonyl carbon of the acetyl group, yielding an acetyl-oxime complex and benzoyl cyanide, as well as the organophosphate acid. Both the acetyl-oxime complex and benzoyl cyanide hydrolyze further to...
produce the same products as the main reaction mechanism, although at a much faster rate\textsuperscript{35}. Because of the speed of this hydrolysis, neither the acetyl-oxime complex nor benzoyl cyanide can continue to be attacked by oxime anions, thereby preventing a chain reaction type of process. It is possible that PBO degradation takes place via an oxime anion attack of another oxime molecule (or anion) at the acetyl carbonyl carbon.

No studies have been conducted on the degradation of PBO or other keto-oximes in an aqueous environment. Therapeutic oximes designed to serve as antidotes to organophosphate poisoning exhibit degradation in aqueous environments; however the oxime functional group in these compounds is attached to a quaternary nitrogen pyridinium ring\textsuperscript{36,40}. This charged ring aids in positioning the molecule at the active site of the inhibited enzyme so that the oxime group can react and subsequently remove the bound organophosphate\textsuperscript{36}. Degradation of these oxime compounds occurs in a significantly different manor than that of the keto-oximes due to this charged ring structure\textsuperscript{41-43}.

It is also possible that diketo-oximes such as PBO degrade in a mechanism similar to Beckmann rearrangement seen for mono-keto oximes. Under basic conditions, monoketo-oximes undergo the reaction mechanism shown in figure 2.2\textsuperscript{44}.

\begin{equation}
\begin{array}{c}
\text{R} - \text{C} - \text{C} - \text{R'} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{R} - \text{C} - \text{C} - \text{OH} \\
\end{array}
\end{equation}

\textbf{Figure 2.2:} General Beckmann rearrangement mechanism for $\alpha$-oximino ketones under basic aqueous conditions.

This type of reaction would only require only a single molecule of oxime. In the case of the diketo-oximes, an acyl carbo-cation and an acyl cyanide would be produced, which would subsequently react further to produce two organic acid molecules.
Solid phase micro-extraction is a technique for collecting and concentrating chemical species from a sample without the use of additional solvents. Solid phase micro-extraction is typically performed using a short (~1 cm) fused silica fiber with a thin layer of solid or semi-solid polymer coating the exterior\(^45\). It is in essence a gas chromatography column turned inside out; the external polymer coating being equivalent to the stationary phase inside of separation columns. It is not surprising then that common SPME fiber polymer coatings are similar in composition to column stationary phases. Some of these common polymers are listed below.

- Polydimethylsiloxane (PDMS)
- Divinylbenzene (DVB)
- Polyacrylate (PA)
- Polyethylene Glycol (PEG)
- Carboxen

Each polymer type differs slightly in polarity and affinity for certain molecular sizes. Non polymeric material is also used, such as Carboxen, which is a carbon based molecular sieve. These various coating materials can be used individually, or several types can be combined to broaden the range of compounds absorbed during the extraction process. Coating thickness can also be adjusted to change the fibers absorptive capacity and usually varies between 10 – 100 \(\mu\)m.

The extraction process begins with sample preparation. If the concentration of absorbable organics in the sample is too high, the polymer coating will swell excessively during the extraction process. Care must be taken to prevent this from happening as is can cause permanent damage to the coating. Samples are often dissolved/diluted in an aqueous solution to lower the concentration of organics present. This can also serve to disperse the sample and increase mass transport to the SPME fiber. If the concentration of the sample in the aqueous solution is excessively low, the addition of various salts and/or pH adjustments can be made\(^{45, 46}\). This will have the effect decreasing the samples water solubility, thus shifting its’ equilibrium towards absorption on the SPME fiber.

The SPME fiber is inserted either directly into the aqueous sample or is positioned in the sample vials’ headspace. Headspace extraction benefits from greatly improved mass transport
(increased diffusion) in the gas phase, as well as improved fiber lifetime. However, headspace extraction is limited to collecting only volatile compounds, as opposed to direct extraction, which can collect non-volatile, larger molecular weight compounds. This extraction process will continue until equilibrium is reached between the solution and the fiber coating. Agitation of the sample during extraction by stirring or ultrasound can decrease equilibration time by increasing mass transport to the polymer. Elevated temperature can also be utilized to speed up mass transport, however this will shift the equilibrium away from absorption to the fiber coating. Once the desired amount of extraction has taken places, the fiber is then removed from the sample and inserted into the heated injector assembly of a gas chromatography system. The sample then quickly desorbs from the fiber coating and is separated and subsequently analyzed by a flame ion detector (FID), mass spectrometer (MS) or other inline detector.

For direct SPME, the equilibrium relationship between the concentration of a species in the fiber coating versus its concentration in the aqueous phase is governed by a distribution coefficient $K$, which can be defined as:

$$K_i \equiv \frac{C_{if}}{C_{is}}$$  \hspace{1cm} 2.1

where $K_i =$ distribution coefficient of species $i$,

$C_{if} =$ concentration of species $i$ in the fiber coating, M

$C_{is} =$ concentration of species $i$ in the aqueous sample. M

It then follows that the total amount of species $i$ extracted should be:

$$n_i = K_iC_{io}\frac{V_fV_s}{K\bar{V}_f+V_s}$$  \hspace{1cm} 2.2

where $n_i =$ total amount of species $i$ in fiber, mol

$V_f =$ volume of fiber coating, L

$V_s =$ volume of aqueous sample, L

$C_{io} =$ concentration of species $i$ in aqueous solution initially, M
For large solution volumes, \( V_s \gg K_i V_f \), and thus the equation can simplify down to \( n_i = K_i V_f C_{io} \). Both of these equations indicate that there will be a direct, proportional relationship between the amount of a species extracted via SPME to its concentration in the sample. The difficulty in performing quantitative calculations for SPME analysis lies in determining the value of the distribution coefficient. For a single species at concentrations well below the saturation concentration for the fiber, its \( K \) value will remain fairly constant. When multiple compounds are present in the sample solutions, a matrix effect will is possible, where one species can interfere with the absorption of another species. This alters \( K \) values and the ranges over which they relatively constant, if at all. This matrix effect also comes into play when selecting a reference or standard compound to add to a sample to improve SPME accuracy and repeatability. By utilizing a structurally identical, yet isotopically different compound from the one being measured in the sample, any matrix effects can be avoided.

2.3 Micro-fluidic mixing

Fluidic flow can be characterized by a number of dimensionless groups, one of which is the Reynolds number, given by the following equation:

\[
Re \equiv \frac{\rho v L}{\mu}
\]

where \( Re \) = Reynolds number,
\( \rho \) = density of fluid,
\( v \) = velocity of fluid,
\( L \) = characteristic length of fluid flow geometry,
\( \mu \) = viscosity of fluid.

The Reynolds number is the ratio of inertial to viscous forces in the fluid flow and can be used to determine which flow regime, laminar or turbulent, is present. Laminar flow is characterized by parallel “streamlines” which do not cross and do not vary with time, while turbulent flow is characterized by local chaotic eddies which are time dependent. For a closed
pipe/channel, the flow will be laminar for \( \text{Re} << 2000 \) and turbulent for \( \text{Re} > 2000 \). At characteristic lengths of several hundred microns or less and at typical flow rates, most liquid flows will be laminar in nature. Due to the nature of laminar flow, mixing of one or more parallel flows in micro scale will occur only via the slow process of diffusion. Countless micro-mixers have been designed for the purpose of decreasing mixing time and increasing mixing efficiency, however they can all be classified as either passive or active devices. Passive mixing devices rely solely on the energy provided by the flow pressure whereas active devices utilize external energy input to operate\(^{49}\). Design constraints of our oxime based micro-sensor require the integrated mixer to be simple, passive device, thus active mixing designs were mostly ignored. Examples of passive mixers include simple T-type contact designs\(^{50}\), cross type fluid focusing\(^{51}\), multiple parallel lamination\(^{52,53}\), repeated stream folding and flattening\(^{54}\), forced fluid contact (Tesla structures)\(^{55}\), ridged channel walls\(^{56,57}\), and Dean vortices in spiral mixers\(^{58}\).
Chapter 3:
Statement of Purpose

A two phase, microfluidic sensor has been previously developed by our group for the detection of organo-phosphate vapors which not only is extremely portable, but also exhibits very high selectivity and sensitivity. This is achieved by using an oxime based chemistry in an aqueous environment to electrochemically detect the compounds of interest. However one of the major drawbacks to this detection scheme is the long term stability of the oxime in solution. Previous work on this oxime electrochemistry indicated that the optimal buffer pH for the aqueous solution was approximately 10. At this pH, the potential change, or response, of the detector to a fixed amount of analyte was maximized. However, sensor response slowly decreases as the aqueous oxime solution ages, by approximately 80% in first 24 hours.

Under current conditions, the oxime solution does not have the stability necessary for real-life sensor applications. The main objectives of this research are the following:

1. Better understand the oxime degradation process, the degradation products, the mechanism by which they are created and the factors that effect the speed of this process.
2. Develop a new liquid medium to store the oxime in. Multiple organic solvents will be evaluated to determine the extent to which they prevent degradation, as well as their compatibility with the existing electrochemical detection method.
3. Study micro-sensor performance of modified oxime solution once beaker scale test have identified the optimal organic solvent.
4. Design and implement a micro-mixer unit integrated with the original micro-sensor. Compare performance of the new integrated device to that of the original sensor.

These steps should reduce or eliminate the oxime degradation without adversely effecting detection performance, allowing these types of sensors to become better suited for real world application.
Chapter 4:
Experimental Apparatus and Procedure

The following section describes the design of the equipment used and the various procedures necessary to carry out the experiments discussed in chapter 5. These procedures include the solid phase micro extraction analytical experiments as well as the beaker scale and micro scale electrochemical test on the oxime solution.

4.1 Solid phase micro extraction of aqueous oxime solution

4.1.1 Extraction setup

Solid phase micro extraction was carried out using disposable 10ml glass vials for sample containment. A gas chromatograph (GC) inlet septum is fitted into the top of the vial provide a gas tight seal which can be punctured by the SPME needle. A water bath is used to maintain the correction extraction temperature for the sample vial. The extraction temperature and stirring rate are then controlled by a hot plate with a digital readout. Finally the SPME fiber holder is stabilized during the extraction period with a standard glassware clap integrated into the hotplate. Figure 4.1 depicts this experimental setup.
Figure 4.1: Solid phase micro extraction experimental setup for extraction of organic compounds directly from oxime solutions.

4.1.2 Gas Chromatograph/Mass Spectrometer configuration

An Agilent 6890N gas chromatograph with a Restek RTX-OPPesticide column (30 m x 250 µm x 0.40 µm) was used in conjunction with an Agilent 5973N mass spectrometer for the SPME analysis. Ultra-pure helium is used as the carrier gas at a constant flow rate of 1 sccm. The GC is set for a splitless injection for 1 minute, after which the injector is purged with helium at 50 ml/min for the rest of the run. The injector assembly is set to 200 °C. The column temperature is slowly increased over the course of the run with the following programmed ramp
up: The column oven is set to 35 °C for 5 minutes, then increased by 5 °C/min to 170 °C, then by 20 °C/min to 250 °C, and finally remaining at this temperature for 10 minutes. The MS is set with a positive electrode bias. To avoid noise produced by atmospheric oxygen, nitrogen and carbon dioxide, the minimum m/z ratio was set at 45. The maximum m/z for the MS scan was set at 195; slightly higher than the molecular weight of the oxime at 191.

4.1.3 Experimental extraction and analysis procedures

All solid phase micro extractions followed the general procedures outlined below.

1. The solution to be analyzed is first prepared and allowed to wait the specified amount of time. The oxime solution is prepared by dissolving 1mg of oxime per 1 ml of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH). This solution is then allowed to sit for a specified amount of time before being sampled for analysis. Calibration solutions are prepared at various concentrations to form a calibration curve and are sampled immediately for analysis.

2. At the designated analysis time, 4 mL of solution is sampled into the 10ml vial and 1.25 g of NaCl (~23.8 wt%) is added to decrease the solubility of organics in solution. A small stir bar is also included in the vial to facilitate mixing.

3. The vial is immediately sealed with the GC septum, and then mixed by hand until the NaCl has completely dissolved.

4. The vial is then placed in a water bath at a temperature of 40 °C, and a stir rate of 450 rpm, and is allowed to equilibrate for 15 minutes.

5. The manual injection SPME fiber holder is equipped with one of the following extraction fibers: Carboxen/PDMS, PDMS/DVB or PA.

6. To clean the fiber and prepare it for the extraction, the fiber needle is set to a depth of 3 cm and inserted into the GC injector port. The GC injector is set to the appropriate conditioning temperature for the fiber being used (300 °C for Carboxen/PDMS, 250 °C for PDMS/DVB, and 280 °C for PA.) The oven temperature is set at 250 °C to prevent contaminates from the cleaning from collecting in the column. The fiber is cleaned in the GC for 15 minutes.
7. Once the fiber has been cleaned, it is quickly removed from the G, the needle depth readjusted to 2.8 cm, and then inserted into the vial containing the sample. At this depth, the fiber is completely submerged in the solution. The extraction is conducted at 40°C and a stir rate of 450 rpm for 50 minutes.

8. Once the extraction period is finished, the GC is prepped for the analysis run. Then the SPME fiber is removed from the sample, immediately set for a depth of 3 cm again and inserted into the injector port. The run is then started at this point. After 4 minutes into the run, the SPME fiber is removed from the injector.

4.2 Beaker scale oxime degradation studies

4.2.1 Beaker electrochemical cell

All beaker scale experiments were conducted using a cyanide ion selective electrode (CN ISE) purchased from Thermo Electron Co. which has a combined liquid-junction reference electrode. The surface of the CN ISE is periodically polished with DI water and an abrasive strip to remove any residue on the surface of the outer (working) electrode. The inner portion of this combined electrode is filled daily with a reference solution pre-made by the same company. The fill port is then covered using Parafilm® to prevent leakage and to hold the electrode assembly in the holder. A custom fabricated Teflon® cap was used to support the electrode over the solution in a 60 mL bottle. This electrochemical cell is placed on a hot plate for stirring only. This experimental setup is illustrated in figure 4.2. The sample solution is added and the electrode is allowed to reach equilibrium. At this point, other solutions may be added to the sample through the other ports in the Teflon® cap.
4.2.2 Experimental procedure (oxime directly in buffer)

All beaker scale experiments followed the general procedures outlined below.

1. Oxime solution to be analyzed is first prepared and allowed to wait the specified amount of time. The 4.75 mM oxime solution is prepared by dissolving 1mg of oxime per 1 ml of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH). This solution is then allowed to sit for a specified amount of time before being sampled for analysis.
2. At the designated analysis time, 10 mL of solution is sampled into the 60 mL electrochemical cell, and the Teflon® cap with the CN ISE is screwed on.
3. The Voltmaster potentiostat is connected to the 2 terminals of the CN ISE and set to measure an open circuit potential.
4. The electrode is allowed to reach equilibrium with the solution as evidenced by a constant solution potential reading. This represents the initial or baseline potential of the solution.
5. To initiate the reaction, 50 µL of a 21 mM solution of acetic anhydride (analyte) in acetone is injected into the cell, resulting in a final analyte concentration in solution of 50 µM.
6. Once the reaction has completed and the solution potential has once again stabilized, the final potential is recorded.
7. Before proceeding to the next experiment, the electrode is washed in DI water to remove any trace cyanide.

4.2.3 Experimental procedure (oxime initially in organic solvent)

The procedure for this section is nearly identical to that of section 4.2.2, with the exception of steps #1-2.

1. Oxime is first dissolved in the organic solvent being tested in a 10 mg Oxime per 1 mL solvent ratio. This solution is then allowed to sit for a specified amount of time before being sampled for analysis.
2. The electrochemical cell is filled with 9 mL of blank buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH) and the Teflon® cap with the CN ISE is screwed on. At the designated analysis time, 1 mL of the oxime/organic solvent solution is added, and thoroughly mixed. This results in a 4.75 mM oxime solution in the electrochemical cell.
4.3 Micro-fluidic sensor studies

4.3.1 Sensor fabrication

The micro-sensor is fabricated by precision machining channels on the surface of small (1 x 1.5 x 0.5 cm) polycarbonate blocks. The liquid channels are 500 µm wide, the gas channels are 1000 µm wide, and all channels are machined to a depth of 100 µm into the polycarbonate surface. Through holes are cut at the ends of all channels to allow for external fluid hook-ups. Figure 4.3 shows the design of both the liquid and gas microchannels.

![Figure 4.3: Top view of liquid (A) and gas (B) polycarbonate micro-channels](image)

The gas and liquid side channels are designed to overlap when both pieces are aligned on top of each other (figure 4.4). Separating the two pieces is a 6 µm thick, track etched polycarbonate membrane with 50 nm pores (SPI-Pore™ Polycarbonate Membrane Filters, Non-Hydrophilic Pore Coating, SPI Supplies). A 40 nm layer of gold is sputtered on to the liquid side of the polycarbonate membrane. This layer is patterned with by shadow mask into two separate regions, for each liquid/gas channel pair, which will function as a working and reference.
electrode. Bonding of all pieces is done via an epoxy (Loctite, 81120) transfer process from a flat PDMS puck to ensure a thin (~6 µm) gas tight seal.

**Figure 4.4:** A cross sectional diagram of a pair of micro-channels (the figure is not to scale). The gas microchannel and the liquid microchannel are aligned over each other and are separated by a nanoporous membrane. The liquid side of the nanoporous membrane is coated with a gold electrode material. (A) Profile along the microchannel. (B) Profile perpendicular to the microchannel.

4.3.2 Integration with gas chromatograph

The completed sensor can be connected by inserting tubing in the pre-drilled through holes. For the liquid side through holes, 1/16 inch tubing is connected to the entrance and exit, and is affixed using epoxy. This tubing can then be connected to an oxime delivery syringe and waste reservoir. For the gas side, capillary sleeves serve as adaptors between the large diameter through holes and the capillary (150 µm I.D., IP deactivated fused silica guard/retention gap columns, Restek) used in the GC. These capillary sleeves are also bonded to the polycarbonate sensor using epoxy. The gas side inlet capillary is then connected to the automatic injector assembly of the GC to provide the sensor with discrete “pulses’ of the analyte within an inert helium carrier gas flow. The automatic injector assembly is the outfitted with a 10µL syringe for sampling the analyte. An integrated low pass filter and 20 times amplifier is connected to the two electrodes of the sensor to remove high frequency noise and boost the sensor response. The output of this amp/filter is connected to the Voltmaster potentiostat, which is again set to measure an open circuit potential.
4.3.3 Experimental procedure (oxime directly in buffer)

All micro-sensor experiments followed the general procedures outlined below.

1. Oxime solution to be analyzed is first prepared and allowed to wait the specified amount of time. The 4.75 mM oxime solution is prepared by dissolving 1 mg of oxime per 1 ml of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH). This solution is then allowed to sit for a specified amount of time before being sampled for analysis.

2. At the designated analysis time, this solution loaded into a syringe and slowly introduced into the liquid channel of the sensor.

3. The sensor, amp/filter and oxime containing syringe are all loaded into the GC oven set at 25°C. The sensor is then allowed to come to equilibrium for 20 – 30 minutes before any analyte is introduced.

4. A small amount of acetic anhydride is put in a GC vial to be used as the analyte during test. The GC automatic injector is then set to sample 1 µL of the headspace of the vial.

5. The GC is set at a 3000:1 split ratio to dilute the acetic anhydride vapor to 0.033% of its initial concentration in the helium carrier gas.

6. When an injection is started, the sampling needle automatically draws in ambient air 6 times to “wash” the syringe. The acetic anhydride vapor is then sampled, injected, diluted by the split flow, and set through the capillary to the sensor as a “pulse”. The electrochemical response of the sensor is then picked up by the external potentiostat. During the test, the oxime solution remains static to maximize the potential response.

7. Once the test has finished, the oven is opened, and fresh oxime solution is again slowly injected into the sensor to remove reaction products and reset the experiment.

4.3.4 Experimental procedure (oxime initially in organic solvent)

The procedure for this section is nearly identical to that of section 4.3.4, with the exception of steps #1-2.
1. Oxime is first dissolved in the organic solvent being tested in a 10 mg Oxime per 1 mL solvent ratio. This solution is then allowed to sit for a specified amount of time before being sampled for analysis.

2. At the designated analysis time, 9 mL of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH) is mixed with 1 mL of the oxime/organic solvent solution, and thoroughly mixed. This results in a 4.75 mM oxime solution for use in the micro sensor.

4.4 Integrated micro-mixer studies

4.4.1 Fabrication of micro-mixer

The integrated micro-mixer channel is machined into a polycarbonate block the identical dimensions of liquid/gas channel blocks (1 x 1.5 x 0.5 cm). Through holes are cut in the micro-mixer block to serve as the inlets for the organic and aqueous liquid streams being mixed. The end of the micro-mixer channel is designed to overlap with the liquid inlet of the sensor when the two blocks are positioned above each other. Figure 4.5 shows the design for the integrated serpentine micro-mixer.
Figure 4.5: The serpentine channel micro-mixer (far right) and its integration into the current microfluidic sensor. Channel dimensions for the micro-mixer are 100 x 500 µm x 46 mm. The basic aqueous buffer and then the organic oxime solution enter the serpentine channel. After mixing, the solution passes to the liquid side of the sensor, where it can be exposed to the analyte vapor.

Using a previously constructed polycarbonate sensor, a liquid outlet is created which drains out the side of the sensor. The original through hole is then sealed with epoxy and is made flush with the top surface of the polycarbonate block. The micro-mixer is then bonded to the liquid side of the sensor using the same epoxy transfer technique used to create the sensor. The inlets/outlets for all liquid and gas streams are then connected to 1/16 inch tubing and bonded with epoxy.

4.4.2 Experimental setup

The organic oxime solution and the aqueous buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH) are delivered to the integrated micro-mixer in a controlled fashion via a syringe pump (Harvard Apparatus PHD 2000). This syringe pump can be equipped with two different size syringes simultaneously, thus allowing the flow rate of each liquid to be controlled by the diameter of the syringe used to deliver it. A 14 mm diameter syringe is used for the aqueous buffer solution, while a 4.5 mm diameter syringe is used for the organic oxime solution, giving approximately a 10:1 ratio of aqueous to organic solution.
Due to the size of the syringe pump setup, the sensor can no longer be connected to the GC to test its performance. Therefore a second syringe pump will be used to supply the analyte vapor to the sensor in a continuous stream instead of a “pulse”. The amp/filter is connected to the two electrodes of the sensor and the output of this is connected to the Voltmaster potentiostat, which is set to measure an open circuit potential.

4.4.3 Experimental procedure

The micro-mixer comparison test was conducted following the steps below.

1. An organic solution of 11 mg oxime per 1 ml of ethanol is created fresh for the test. This solution is loaded into the 4.5 mm diameter syringe.
2. The 14 mm diameter syringe is loaded with 25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH.
3. Due to syringe pump limitations, the minimum reliable plunger speed is roughly 30 µm/min. This speed gives an aqueous buffer solution flow rate of 5.0 µL/min and an organic oxime solution flow rate of 0.5 µL/min.
4. A syringe containing acetic anhydride vapor dilute to 100 ppb using ambient air is loaded into the second syringe pump, which is set to provide a gas flow rate of 1 mL/min.
5. The open circuit potential of the sensor is monitored to determine when the system has reached a steady-state equilibrium. This potential corresponds to the initial or baseline reading.
6. The test is then started by initiating the flow of analyte vapor. This gas stream is flows through the sensor for 100 seconds and then turned off. The sensor is then allowed to return to its initial potential reading. The liquid syringe pump remains operational and pumping fluid at the designated flow rate during the entire test.
7. The comparison test is conducted by flushing out the system with DI water, and preparing fresh organic oxime and aqueous buffer solution as per step #1.
8. The aqueous buffer will be combined with the organic oxime solution in a 10:1 ratio again, however this time they will be mixed externally by hand.
9. This pre-mixed solution is then loaded into a syringe and the syringe pump is then set to deliver the fluid at a flow rate of 5.5 µL/min.

10. The rest of the comparison test is then carried out by following steps 4 – 6.
Chapter 5: Experimental Results and Discussion

5.1 Oxime degradation

The apparent degradation of the oxime PBO in the aqueous buffer solution manifest itself as an increase in both the initial (before analyte exposure) and final (after analyte exposure) potential of the system (figure 5.1).

![Figure 5.1: Potential response decrease over 24 hour period.](image)

Both changes are a result of increased cyanide ion concentration in the solution caused by the breakdown of PBO. However, due to the logarithmic nature of the concentration versus
potential relationship, the increase in cyanide ion concentration raises the initial potential substantially more than the final potential, thus having the end effect of decreasing the magnitude of the sensors potential response to an analyte. Using the potential change versus acetic anhydride concentration data from Oh, I. et al, we find the logarithmic relationship between cyanide concentration and potential change is given by:

$$\Delta E = 63 \log[CN^-] + 427$$

where $\Delta E = \text{magnitude of potential change of solution, mV}$

$$[CN^-] = \text{concentration of cyanide ions in solution, M}$$

Beaker cell experiments monitoring solution potential over an extended period of time show the effects of oxime degradation on solution baseline potential. Converting potential change measurements to cyanide concentrations we get figure 5.2.

**Figure 5.2:** Degradation of 5 mM PBO in basic buffer at various pH values.
Oxime degradation occurs at all pH levels tested, however the rate of degradation increases at higher pH. Below pH 9, PBO solubility in the aqueous solution dramatically decreases, and we are unable to create solutions at the standard 5mM concentration. Decreasing the oxime concentration in the buffer solution to 2.5 mM, we observe a decrease in the rate of degradation (figure 5.3).

Figure 5.3: Degradation comparison of 5mM versus 2.5 mM PBO concentration in basic buffer at pH 10.

In the pH range looked at, PBO anion concentrations are one to three orders of magnitude higher than hydroxide ion concentrations. This information indicates that the degradation mechanism possibly involves the PBO anions either reacting with hydroxide ions or even with each other. One thing that is clear is that cyanide ions are being created during the degradation process. Because the degradation takes place when PBO is in its anion form, dissolving it in an organic solvent will prevent this from occurring. Acetonitrile was found to be the ideal organic
solvent for dissolving oxime for GC injection, as it gave the “cleanest” spectrum. The first thing to note about this spectrum is that oxime is eluted in 2 peaks, possibly due to some sort of oxime dimer being formed, although MS results do not show any evidence of this. It is also important to note that benzoic acid, benzoyl cyanide and acetic acid also appeared in this spectrum. These compounds may have been present originally in solid oxime powder; however it is also possible that they were created by the oxime spontaneously decomposing in the high temperatures of the GC injector. Other compounds of note are a large peak most likely corresponding to toluene as well as several small nitrile peaks, most likely related to the solvent used. Because this was a direct injection of solution, the MS could not be turned on until 3 minutes into the run, to avoid exposing the filament to the large solvent peak at the beginning. Compounds eluted in the first 3 minutes of the run were therefore not detected.

To understand this decomposition mechanism better, solid phase micro-extraction was performed on the basic aqueous buffer solution to determine what other compounds were being formed. A polyacrylate (PA) coated fiber was used for all the extractions because its polar nature is ideal for capturing organic acids which may be produced during the degradation process. Unfortunately, hydrogen cyanide is too light to be significantly absorbed by the fiber and will not be retained (concentrated) by the GC column. A blank run consisting of only basic aqueous borate buffer (pH 10) was initially examined via SPME to serve as a sort of baseline and to determine what contaminants may be present. Several compounds identified as 1,2-benzenedicarboxylic acid esters were found, as well as benzophenone, diphenylamine and diphenylether. Several peaks identified as benzene and one as toluene were also found. These compounds will be ignored when analyzing the SPME results, unless their elution time changes significantly.

When SPME was performed on the basic aqueous buffer with oxime, we get the following identifiable peaks (table 5.1).
Table 5.1: Identifiable peaks (ignoring known contaminants) from SPME of a 5 mM solution of oxime in aqueous buffer (pH 10) performed 1 hour after solution preparation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Elution Time (min)</th>
<th>Peak Area (x100000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>4.09</td>
<td>14</td>
</tr>
<tr>
<td>Toluene</td>
<td>5.44</td>
<td>540</td>
</tr>
<tr>
<td>N-Hydroxy Benzamide</td>
<td>19.25</td>
<td>12</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>20.38</td>
<td>150</td>
</tr>
<tr>
<td>Benzyol cyanide</td>
<td>20.54</td>
<td>275</td>
</tr>
<tr>
<td>1-Phenyl 1,3-butadione</td>
<td>27.92</td>
<td>92</td>
</tr>
<tr>
<td>2-Isonitrosoacetophenone</td>
<td>28.35</td>
<td>6</td>
</tr>
<tr>
<td>PBO minus oxygen</td>
<td>29.30</td>
<td>4</td>
</tr>
<tr>
<td>PBO (1st peak)</td>
<td>34.57</td>
<td>1665</td>
</tr>
<tr>
<td>PBO (2nd peak)</td>
<td>35.05</td>
<td>566</td>
</tr>
</tbody>
</table>

Similar to the spectrum from the direct injection of oxime, we find acetic acid, benzoic acid, benzoyl cyanide, and 2 oxime peaks. We also find N-hydroxy benzamide, 1-phenyl 1,3-butadione, 2-isonitrosoacetophenone, and a compound whose weight of 175 corresponds to that of oxime minus an oxygen atom. It is reasonable to believe that the compound with a weight 175 is the imine version of PBO, in which the hydroxyl group is replaced by a hydrogen group. If this is the case, 1-phenyl 1,3-butadione, 2-isonitrosoacetophenone and the imine are all oxime fragments where a portion of the molecule is replaced with a hydrogen atom and it is possible that this type of substitution is occurring in the aqueous solution. N-hydroxy benzamide on the other hand is not likely to have formed due to a simple substitution reaction. The most probable route of formation for this compound would be a rearrangement of the nitrogen from the number 2 to the number 1 carbon, followed by the loss of the remaining 3 carbon chain. Acetic acid, benzoic acid, and benzoyl cyanide are products or intermediates in the reaction mechanism seen in figure 2.1, and could all possibly be involved in a similar mechanism for the degradation of the oxime. However, any benzoyl cyanide present in the solid oxime or created during the degradation would be quickly hydrolyzed to form benzoic acid; therefore most of the benzoyl cyanide eluted must have come from the spontaneous decomposition of the oxime in the GC.
injector inlet. The data from the direct injection of oxime would indicate that this spontaneous decomposition is also responsible for some of the acetic acid and benzoic acid seen, as well as most of the toluene.

Monitoring the oxime degradation over a period of several days we see the following trends with regards to formation (figure 5.4) or destruction (figure 5.5) of compounds.

![Figure 5.4: Compounds which exhibit increases in peak area over time. Normalized to initial peak area.](image)

**Figure 5.4:** Compounds which exhibit increases in peak area over time. Normalized to initial peak area.
Figure 5.5: Compounds which exhibit decreases in peak area over time. Normalized to initial peak area.

As expected, the first oxime peak decreases over time, however the second oxime peak increases dramatically. It is possible this is due to further oxime dimerization while in the aqueous solution. Toluene, which is most likely a created by oxime decomposition in the GC injector, also decreases, seemingly confirming this assumption. Most of the other compounds, which the exception of 1-phenyl 1,3-butadione, increase over time, indicating that they are all possible oxime degradation products. Due to the low molecular weight of acetic acid, the column does not retain this compound very well. As a result, the acetic acid peak is extremely susceptible to variability in the manual injection process of the SPME fiber, with elution times varying by up to a second. This could explain the lack of a strong increasing trend in the acetic acid peak area seen.
It appears that the oxime degradation occurs both through a nucleophilic attack, such as a hydroxyl or oxime anion, as well as by a rearrangement of the nitrogen atom in a Beckmann rearrangement type of process, as evidenced by the production of N-hydroxy benzamide.

5.2 Beaker scale degradation analysis

To prevent degradation from occurring, it is obvious that the oxime must not be allowed to dissociate until the moment it is needed in the sensor. This will be accomplished by initially dissolving PBO in an organic solvent, then combining this organic oxime solution with the basic aqueous buffer just prior to use in the sensor. The criteria for the organic solvent are that it should prevent dissociation of the oxime, be miscible in the buffer, and be able to significantly concentrate the oxime to prevent increasing the total volume of solution needed.

The following five organic solvents were chosen based on their miscibility with water: ethylene glycol, ethanol, acetone, acetonitrile, dimethyl sulfoxide (DMSO). Rough solubility test indicated that all organic solvents had much higher PBO solubility than water, with the exception of ethylene glycol (table 5.2).

Table 5.2: Oxime solubility limits in various solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Water (pH&gt;9)</th>
<th>Ethylene Glycol</th>
<th>Ethanol</th>
<th>Acetone</th>
<th>Acetonitrile</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBO Solubility (per mL solvent)</td>
<td>~1 mg</td>
<td>20 mg</td>
<td>450 mg</td>
<td>500 mg</td>
<td>440 mg</td>
<td>400 mg</td>
</tr>
</tbody>
</table>

In general, PBO is 400 – 500 times more soluble in organic solvents then the basic aqueous buffer solution. A concentrated organic oxime solution not only minimizes the increase in total solution needed, it also reduces the organic solvent to aqueous solvent ratio needed to reach a final PBO concentration of 5 mM. This is helpful in minimizing the interference of the organic solvent on the electrochemical performance of the system.

Each solvent will be tested in beaker scale experiments first to determine which to use in the actual microfluidic sensor. The solvents will be evaluated on their ability to suppress the
degradation of PBO and the degree to which they interfere with the current electrochemical detection method. PBO is initially dissolved in the organic solvent at a 50 mM concentration. This organic oxime solution is then mixed with the basic aqueous buffer at t = 0 or 24 hours after it was initially dissolved. The mixing is done at a 9:1 ratio of aqueous buffer to organic oxime solution, respectively. This results in a final PBO concentration of 5 mM for all test solutions. The 9:1 ratio is chosen rather arbitrarily for convenience; at higher ratios the effects of the organic solvent on the electrochemistry decrease; however it becomes increasingly difficult for a microfluidic device to correctly meter out the two fluids at the precise ratio.

Once the organic oxime solution has been mixed with the basic aqueous buffer solution, the potential (as measured with the CN ISE) is allowed to equilibrate. At this point, the analyte is injected into the beaker cell and the potential is again allowed to equilibrate. The difference in potentials is the potential response (ΔE) of the system to the analyte, and is a critical parameter in judging sensor performance. Table 5.3 shows the effects of the various solvents on ΔE measure in the beaker cell experiments.

**Table 5.3:** Effects of various solvents on beaker cell potential response (ΔE).

<table>
<thead>
<tr>
<th>Organic Solvent</th>
<th>0 Hour ΔE (mV)</th>
<th>24 Hour ΔE (mV)</th>
<th>Degradation of ΔE (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Organic Solvent</td>
<td>154</td>
<td>92</td>
<td>62 ± 9</td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>157</td>
<td>145</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>Ethanol</td>
<td>150</td>
<td>149</td>
<td>1 ± 10</td>
</tr>
<tr>
<td>Acetone</td>
<td>145</td>
<td>141</td>
<td>5 ± 10</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>113</td>
<td>113</td>
<td>0 ± 5</td>
</tr>
<tr>
<td>DMSO</td>
<td>74</td>
<td>66</td>
<td>9 ± 14</td>
</tr>
</tbody>
</table>

In the “no organic solvent” case, PBO is directly dissolved in the buffer at t = 0 hours. Analyte is then injected into the solution at t = 0 or 24 hours and the resulting ΔE is measured as usual. As mentioned previously, degradation manifests itself as a decrease in ΔE over time. Compared to the “no organic solvent” case, all organic solvents tested greatly suppressed PBO degradation.
Electrochemical interference manifests itself as a decrease in $\Delta E$ compared the “no organic solvent” case at $t = 0$ hours. Most solvents tested do interfere with the electrochemistry to varying degrees, except for ethylene glycol, which may have a slight enhancing effect on $\Delta E$. It is clear from this data that the best solvents for use in further experiments are ethylene glycol and ethanol. The differences in $\Delta E$ for each solvent can be clearly seen in Figure 5.6, with ethylene glycol and ethanol closest to the original “no organic solvent” system.

![Typical potential response curves for each solvent tested. Curves normalized to baseline potential. All test performed in a borate buffer solution at pH 10.](image)

**Figure 5.6:** Typical potential response curves for each solvent tested. Curves normalized to baseline potential. All test performed in a borate buffer solution at pH 10.

Sample response curves of each solvent tested at $t = 0$ hours (as well as $t = 24$ hours) show a decrease in the slope of the potential response curve during the reaction in comparison to the “no organic solvent” case (figure 5.6). Acetone in particular has a large negative effect on the rate of cyanide formation, indicating that acetone interferes greatly in the oxime-analyte reaction or the subsequent mass transport of $\text{CN}^-$ to the electrode. Although ethylene glycol and
ethanol do not decrease the rate of cyanide formation as much as acetone, this aspect of solvent – electrochemistry interaction was investigated further during the microfluidic sensor test. Additionally acetone was not considered as a possible solvent due to its incompatibility with the polycarbonate sensor material.

The final aspect to study is the effect of solvents on the optimal operating pH of the system. Previous work in the group has shown that the optimal pH for PBO directly dissolved into the aqueous buffer is approximately 10. Experiments with ethylene glycol and ethanol show the same behavior where the potential response, $\Delta E$, is maximized at pH 10 (figure 5.7). However it is interesting to note that the addition of an organic solvent into the system reduces the effect pH changes have on $\Delta E$, especially at higher pH values. In real world use, this will benefit the sensor by making it less susceptible to pH changes caused by highly acidic or basic analyte vapors.

Figure 5.7: Potential response ($\Delta E$) versus aqueous buffer pH for various solvents.
5.3 Microfluidic Sensor Performance

Beaker scale tests had the advantage of quickly and easily evaluating a large variety of electrochemical detection systems. However, variability in testing conditions, such as mixing speed and stir bar location, as well as all the variability associated with the manual analyte injection method make beaker scale test only a rough indication of electrochemical performance. To fully evaluate the solvents chosen in the previous experiments, we must move to the actual microfluidic sensor platform and continue testing.

As mentioned previously, the sensor is connected to an automatic injector assembly unit on a gas chromatograph. This allows for much more precise, repeatable analyte injection volume and injection speed. The automatic injector is capable of diluting injections by nearly 4 orders of magnitude, to a maximum of 0.0133% of the original sample concentration. A 1 ml/min flow rate of the carrier gas Helium is used to pass the analyte sample to the sensor in a focused “pulse”. A typical analyte “pulse” as seen through a flame ionization detector (FID) connected in place of the sensor is shown in figure 5.8.

![Typical analyte pulse created by automatic injector assembly of GC, as measured through a flame ionization detector (FID). The y-axis quantity is a measure of electrical signal output by the FID.](image-url)

**Figure 5.8:** Typical analyte pulse created by automatic injector assembly of GC, as measured through a flame ionization detector (FID). The y-axis quantity is a measure of electrical signal output by the FID.

Both the pulse height (concentration) and width (exposure duration) can be reliably reproduced using this GC equipment. Pulse height is controlled by the amount of dilution of the...
analyte sample, while pulse width remains at approximately 15 ms (measured at half the pulse height).

For all sensor experiments, the concentration of the analyte pulse at its maximum “height” was held constant at 1 ppm and the pulse width (at half height) was held at approximately 15 ms. Similar to the beaker scale test, the organic oxime solution is only mixed with the basic aqueous buffer just prior to testing. As mixing liquids on the micro-scale is difficult, we avoid possible incomplete mixing by doing this externally of the sensor. The resulting test solution is then quickly introduced into the sensor, then allowed to equilibrate at static (zero) liquid flow rate, while carrier gas remains flowing in the gas channel.

![Figure 5.9: Typical potential response curves for ethylene glycol, ethanol and “no organic solvent” systems as measured from the microfluidic sensor.](image)

The analyte pulse reaches the sensor at $t = 5$ s, crosses the polycarbonate membrane and begins to react with the PBO on the liquid side of the gas-liquid interface. The gold electrode, also on the liquid side of the liquid – gas interface, registers this cyanide ion build up, causing a sharp increase in potential. Once the analyte pulse has passed through the sensor the reaction
ends and diffusion of cyanide ions away from the electrode slowly lowers the potential back to the baseline value. Figure 5.9 shows the results of exposing the sensor to an analyte pulse. As we saw in the beaker scale experiments, the addition of an organic solvent decreases the potential response of the sensor when compared to the “no organic solvent” case, roughly 20% less for ethylene glycol and 40% less for ethanol. This is more severe a decrease than was observed for the beaker scale experiments, however the lack of active mixing in the microchannel may have played a part in this.

Without active mixing, the organic solvent molecules may have a greater shielding effect on the electrode; not allowing the cyanide ion to reach the electrode before diffusing into the bulk of the fluid. The results of these sensor tests also show that the addition of ethylene glycol seems to have increased the diffusivity of cyanide ions. Higher cyanide ion diffusivity will deplete the concentrated layer of cyanide ions at the liquid – gas interface quicker. This negatively affects sensor performance, as the potential response increases the more cyanide ions are allowed to concentrate near the electrode on the liquid – gas interface.

Figure 5.10: Typical first derivative curves for ethylene glycol, ethanol and “no organic solvent” systems as measured from the microfluidic sensor.
More information can be derived from these set of sensor tests by taking the first
derivative of the potential data. Before the derivative is calculated however, the data must go
through a high frequency noise reduction algorithm, as this noise would be magnified in the
derivative signal. The result of this manipulation is shown in figure 5.10. These curves
represent the rate at which cyanide ions reach the electrode. As the reaction occurs virtually in
the same location as the electrode surface, this could reasonably be interpreted as the rate of
cyanide ions produced in the oxime – analyte reaction. We see that both ethylene glycol and
ethanol decrease the rate of cyanide formation compared to the “no organic solvent” case, by
roughly 30% and 20%, respectively. It is possible that the introduction of an organic solvent
shifts the dissociation equilibration towards the oxime molecule, reducing the amount of oxime
anion in solution, and thus lowering the reaction rate. It is interesting to note that although the
ethylene glycol system has a larger potential response compared to the ethanol system; it has the
lower rate of cyanide ion formation. For this to occur, the reaction must take place over a longer
period of time. This is in fact the case, as we find that the peak width (at half maximum height)
of the differentiated signal is 0.86 s for ethylene glycol, compared to 0.54 s for ethanol, and 0.61
s for the “no organic solvent” case. In comparison to the “pulse” width as seen through the FID,
the peak width of the differentiated potential signal is roughly 30 times longer. Although the
exact cause of this increased response time is unknown, several factors could be responsible.
The analyte pulse may broaden as it passes from the small diameter capillary to the larger
diameter sensor channel. Other factors of sensor channel geometry may also cause broadening
of the pulse. Mass transport across the membrane and the reaction rate with the oxime may also
introduce lag effects which ultimately lengthen the response time of the potential signal.

If the vapor to be analyzed contained more than one species of reactive organophosphate,
a GC column could be used to perform a separation on the gas sample. Each species of
organophosphate would exit the column as a sharp “pulse” after a species specific retention time
based on molecular weight and column – molecule interactions. The smaller the width of each
signal peak produced by the sensor, the easier it becomes to distinguish multiple peaks, and the
easier it becomes to identify multiple organophosphate species. Therefore it is desirable for the
peak width of the differentiated signals from the sensor to be as small as possible. It is this final
criterion that leads us to conclude that ethanol is the most ideal organic solvent for future use in
this microfluidic sensor.
The height of the differentiated potential signal is related to the rate at which cyanide is produced. Increasing the rate of reaction between the oxime and analyte should compensate for the peak height reduction seen when using ethanol. Utilizing the high solubility of the oxime in ethanol, we can double the concentration in solution, raising it to 10 mM in the mixture. The effects of this change on the potential response of the sensor can be seen in figure 5.11.

Figure 5.11: Typical potential response curves for 5mM and 10mM Oxime solutions using ethanol, as well as the “no organic solvent” systems (5mM oxime concentration) as measured from the microfluidic sensor.

The increase in the oxime concentration does in fact boost the potential response of the sensor. With a concentration change from 5 mM to 10 mM, the average potential response more than doubles; from 8.8 mV to 19.5 mV, a 120% increase. However, looking at the differentiated signal we see the rate of cyanide formation remains unchanged (figure 5.12).
Figure 5.12: Typical first derivative curves for 5mM and 10mM Oxime solutions using ethanol, as well as the “no organic solvent” systems (5mM oxime concentration) as measured from the microfluidic sensor.

The lack of change in the cyanide formation rate can be explained if we look at oxime solubility in the aqueous buffer. Solubility in the basic aqueous buffer is highly dependent on pH. At pH values below 9, the oxime solubility begins to drop off, and by pH 7 the oxime is nearly insoluble. This coincides well with the pKₐ of the oxime, so we can conclude that its solubility in aqueous mediums is most likely controlled by the ease at which it can dissociate and form the oxime anion. In organic solvents this is not the case, as the oxime does not dissociate in these mediums. Upon mixing the organic oxime solution with the basic aqueous buffer, some of the oxime molecules dissociate to form anions based on the pH of the buffer. It is possible the despite the increasing in the oxime concentration in the organic solvent, no additional oxime anions can be formed because of the solubility limit in the aqueous solution.

The cause of the large increase in potential response observed in figure 5.10 was actually due to an increase in the total reaction time, as see as an increase in the peak width (at half max
height) of the differentiated signal in figure 5.12. Peak width doubled from 0.54 s at the 5 mM oxime concentration to 1.07 s at 10 mM oxime (both using ethanol).

5.4 Integrated Micro Mixer

The previous tests with the micro sensor relied on the organic oxime solution being mixed with the basic aqueous buffer on the macro scale, externally from the sensor. This assured that the solutions were well mixed before being introduced into the device, ensuring reproducible results. Mixing in the current microfluidic sensor design is rather poor, due to the laminar nature of the fluid flow. In order for the final device to properly mix these two solutions, a specialized integrated micro-mixer unit must be added to the design. In laminar flow conditions, diffusive mixing becomes the major, if not sole, mechanism of mixing. By employing a serpentine channel type mixer we can substantially increase mixer length, thereby increasing the residence time of the fluid in the mixer, ensuring that there is sufficient time for complete diffusive mixing to occur.

The extent of diffusive mixing increases with increased fluid residence time, and consequently decreased fluid flow rate. Additionally sensor performance also increases with decreased fluid flow rate. This is due to more cyanide ions accumulating in the sensor before being flushed out, increasing the potential response. All previous micro-sensor test were conducted at zero (or static) liquid flow rate to maximize cyanide ion accumulation and potential response. Since the mixer and sensor both benefit from lower liquid flow rates, the integrated mixer – sensor device should have a maximum operational liquid flow rate, above which the performance of the device decreases considerably. To evaluate the effectiveness of the micro-mixer it is only necessary to compare the sensor performance of the integrated device to the original device with the two solutions pre-mixed externally. The test will take place at a single, sufficiently low flow rate, so that we avoid significantly reducing micro-sensor performance due to cyanide ion flush out. This will set a maximum operating liquid flow rate for the integrated device. A syringe pump is used to dispense the solutions, at a 10:1, basic aqueous buffer to organic oxime solution flow rate, respectively. The concentration of the oxime in the final solution will remain 5 mM. A syringe pump will be used to dispense the fluids and will operate
near its lowest reliable speed, giving a total liquid flow rate of 5.5 µL/min. For the integrated mixer test, a flow rate ratio of 10:1 gives flow rates of 5.0 µL/min of aqueous solution and 0.5 µL/min of organic solution. Due to equipment size constraints, the syringe pump cannot be run in conjunction with the automatic injector of the GC. Therefore a second syringe pump will be used to supply the analyte vapor to the sensor. The results of this comparison test can be seen in figure 5.13.

![Figure 5.13](image)

**Figure 5.13:** Micro-sensor performance with integrated micro-mixer in comparison to micro-sensor with mixing performed externally. In both cases, the total liquid flow rate is 5.5µL/min and the gas flow rate was 1ml/min. The gas is 100ppb concentration acetic anhydride vapor, and the flows is turned on at t = 50s and off at t = 200s.

Although this test method is not as reproducible as the automatic injector test method, you can clearly see that potential response (ΔE) for both cases is nearly identical. In fact the potential curves are very similar during the entire exposure period between 50 – 200 s. This indicates that the integrated micro-mixer was successful at completely mixing the two solutions.
at the flow rate used. This can be further confirmed if we look at the estimated time required for complete diffusive mixing of two parallel laminar streams, given by equation 5.1:

\[
t = 0.5 \frac{L_{mixing}^2}{D}
\]  

5.1

where \( t \) = time for complete diffusive mixing, s

\( L_{mixing} \) = length over which the mixing occurs, 100 µm

\( D \) = diffusivity of one fluid in the other, ethanol – water diffusivity cm²/s

This gives an estimated time for complete mixing of approximately 6 s. The actual residence time of the fluids traveling through the mixer at 5.5 µL/min is roughly 25 s. It is clear that at the flow rate used in the test, the two solutions had more than enough time to completely mix. In practice the mixer will not be used in this manner. When the micro-sensor is being exposed to a vapor sample, the liquid will remain static, ensuring the maximum possible potential response. While this takes place, stationary fluid in the serpentine micro-mixer mixes via diffusion, which should have more than enough time to mix completely. Between exposures, when the sensor must be flushed out to remove cyanide build up, the fully mixed fluid residing in the serpentine micro-mixer flows to the micro-sensor. The fluid in the mixer is replaced with unmixed solution which will again mix (at static flow rate) while the sensor operates.
Chapter 6:  
Conclusions and Recommendations

6.1 Conclusions

The oxime based electrochemical sensor our group has developed to selectively detect toxic organophosphates and other reactive acid anhydrides suffers from a long term stability issue. In the basic, aqueous buffer solution, the dissolved oxime PBO slowly decomposes over a period of several days. This decomposition process releases cyanide ions which raise the baseline signal of the sensor and reduce its sensitivity to the analyte. The rate of degradation is increased with at higher hydroxide concentration as well as increased oxime concentrations. Solid phase micro-extraction performed on the aqueous oxime solution shows several other possible degradation products, including acetic acid, N-hydroxy benzamide, benzoic acid, benzoyl cyanide, 1-Phenyl 1,3-butadione, 2-Isonitrosoacetophenone and an imine derived from the oxime. Judging from the compounds produced and their increased concentration over time, it is reasonable to conclude the oxime degradation produces cyanide via a nucleophilic attack by a hydroxide (or possibly another oxime) anion. The oxime also decomposes by a rearrangement reaction similar to a typical Beckmann rearrangement, which does not yield any cyanide.

Dissolving the PBO in an organic solvent proved to slow the degradation process significantly. The use of the organic solvent prevents the oxime anion from forming and stops hydroxide ions from attacking it. This organic solution could be added, when needed, to the aqueous buffer for use in the sensor. The aqueous to organic solutions were mixed in a 10:1 ratio which minimized the amount of organic solvent while maintaining the original 5 mM concentration of oxime. From the beaker scale test performed, it was determined that ethanol and ethylene glycol were the best solvents based on their ability to prevent degradation as well as their compatibility with the electrochemical detection method. Combining the existing oxime based micro-sensor with an automated GC injector allows for very accurate testing of the effects of the organic-aqueous solvent mixture on the system. Examining the solution potential and the rate of change in solution potential in response to a pulse of analyte we find that both ethanol and
ethylene glycol lower the rate of formation of cyanide ions. Total response time is slightly decreased when using ethanol and increased when using ethylene glycol, most likely due to mass transport effects. These results indicate that ethanol is the optimal solvent for use with the existing oxime micro-sensor. As the organic solvents allow a higher concentration of oxime to be added to the aqueous solution than directly dissolving would, the sensor was also tested using a 10 mM oxime solution prepared with ethanol. The magnitude of the potential response for this concentration was nearly double the 5 mM response; however this comes as a result of a longer response time and not a faster cyanide production rate.

Beaker and micro scale studies performed on the organic solvents required them to be thoroughly mixed with the aqueous solutions prior to exposure to an analyte. For the sensor to be used in a real world application, it was necessary for this mixing to be done automatically and with the whole process occurring on the micro-scale. A serpentine channel mixer was designed to seamlessly integrate with the current micro-sensor using the same polycarbonate material and fabrication techniques. Such a mixer works solely through diffusion, and operates at very low flow rates. Both the sensor and mixer operate more effectively the lower the liquid flow rate, and for total liquid flow rates (10:1 aqueous to organic ratio) of less than 5.5 µL/min, the sensor response is identical to the case where mixing is done externally on the macro scale.

6.2 Recommendations

To better understand the degradation mechanism as well as obtain reaction kinetic parameters, the accuracy of the SPME technique must be increased. Isotopically label PBO cannot be used as a reference compound as it will suffer the same degradation process as the original oxime. Care must be taken to select an isotopical compound which will not decompose or react further, either in solution, or during the analysis process. Benzoic acid would be the ideal species to choose as it is present in large amounts and is not likely to further react. Even with the use of an isotopic standard, the number of compounds created during the degradation of oxime causes a strong matrix effect that complicates SPME spectrum quantification.

Reverse phase high performance liquid chromatography (RP - HPLC) can be used instead of gas chromatograph (GC) to analyze the compounds extract via SPME. By using HPLC, exposure of the sample to high temperatures can be avoided, thus preventing “false” oxime
degradation products. As the separation process takes place by a different process than that of a GC, new compounds may be seen which were previously obscured by other peaks or were not retained by a GC column. It is extremely important that neither phase used for the RP-HPLC react with the oxime or its products. For example, an aqueous ammonium formate buffer phase combined with an acetonitrile or methanol organic phase may work well for this type of separation and analysis technique.

Optical analysis methods can be used to track the concentration of both oxime and cyanide in solution. The PBO anion, which is found in basic aqueous solutions, absorbs ultraviolet (UV) light at approximately ~260 nm, however this absorbance peak is broad and extends into the visible range, giving these solutions a characteristic yellow color. PBO dissolved in an organic solvent does not dissociate into ions and absorbs light in a more narrow range with a maximum at ~240 nm. The loss of oxime in either solution could be monitored via a UV-vis spectrometer, as long as the high UV absorbance of the solution does not overwhelm the detection mechanism. Cyanide ions absorb infrared light at a frequency of approximately 2640 cm\(^{-1}\) and thus their production can be monitored via fourier transform infrared spectroscopy (FTIR). Preliminary work on this detection method revealed that in order to detect cyanide in the concentrations being produced, a multi-pass IR beam would be needed to achieve sufficient absorbance for a cyanide peak to be seen.

A possible improvement which could be made in the integrated micro-mixer and sensor device would be the addition of cyanide “filter”. A small porous region of fine silver particles can be placed in the liquid flow channel shortly before entering the actual sensor. Cyanide binds very strongly to silver and any present due to degradation would be removed from the liquid, thus preventing it from interfering with cyanide produced in the detection process. The difficulty lies in the creation of this silver “filter” within the flow channel in a way that is compatible with existing fabrication techniques and prevents silver particles from being flushed out of the system and contaminating the gold electrodes.
References


Appendix A: Additional Figures

**Figure A.1:** A 5 mM solution of PBO in acetonitrile directly injected into the GC at a split of 100 (a 101:1 dilution).
Figure A.2: SPME of the basic aqueous buffer solution without dissolved PBO.
Figure A.3: SPME of a 5 mM PBO dissolved in basic aqueous buffer sampled immediately after it has been prepared.
Figure A.4: SPME of a 5 mM PBO dissolved in basic aqueous buffer sampled 1 hour after it has been prepared.
Figure A.5: SPME of a 5 mM PBO dissolved in basic aqueous buffer sampled 12 hours after it has been prepared.
Figure A.6: SPME of a 5 mM PBO dissolved in basic aqueous buffer sampled 28 hours after it has been prepared.
Figure A.7: SPME of a 5 mM PBO dissolved in basic aqueous buffer sampled 72 hours after it has been prepared.
Appendix B: Experiment Procedures

Solid phase micro-extraction and analysis procedures

All solid phase micro extractions followed the general procedures outlined below.

1. The solution to be analyzed is first prepared and allowed to wait the specified amount of time. The oxime solution is prepared by dissolving 1mg of oxime per 1 ml of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH). This solution is then allowed to sit for a specified amount of time before being sampled for analysis. Calibration solutions are prepared at various concentrations to form a calibration curve and are sampled immediately for analysis.

2. At the designated analysis time, 4 mL of solution is sampled into the 10ml vial and 1.25 g of NaCl (~23.8 wt%) is added to decrease the solubility of organics in solution. A small stir bar is also included in the vial to facilitate mixing.

3. The vial is immediately sealed with the GC septum, and then mixed by hand until the NaCl has completely dissolved.

4. The vial is then placed in a water bath at a temperature of 40 °C, and a stir rate of 450 rpm, and is allowed to equilibrate for 15 minutes.

5. The manual injection SPME fiber holder is equipped with one of the following extraction fibers: Carboxen/PDMS, PDMS/DVB or PA.

6. To clean the fiber and prepare it for the extraction, the fiber needle is set to a depth of 3 cm and inserted into the GC injector port. The GC injector is set to the appropriate conditioning temperature for the fiber being used (300 °C for Carboxen/PDMS, 250 °C for PDMS/DVB, and 280 °C for PA.) The oven temperature is set at 250 °C to prevent contaminates from the cleaning from collecting in the column. The fiber is cleaned in the GC for 15 minutes.

7. Once the fiber has been cleaned, it is quickly removed from the G, the needle depth readjusted to 2.8 cm, and then inserted into the vial containing the sample. At this depth, the fiber is completely submerged in the solution. The extraction is conducted at 40°C and a stir rate of 450 rpm for 50 minutes.
8. Once the extraction period is finished, the GC is prepped for the analysis run. Then the SPME fiber is removed from the sample, immediately set for a depth of 3 cm again and inserted into the injector port. The run is then started at this point. After 4 minutes into the run, the SPME fiber is removed from the injector.

Beaker scale electrochemical studies (oxime directly in buffer)

All beaker scale experiments followed the general procedures outlined below.

1. Oxime solution to be analyzed is first prepared and allowed to wait the specified amount of time. The 4.75 mM oxime solution is prepared by dissolving 1mg of oxime per 1 ml of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH). This solution is then allowed to sit for a specified amount of time before being sampled for analysis.

2. At the designated analysis time, 10 mL of solution is sampled into the 60 mL electrochemical cell, and the Teflon® cap with the CN ISE is screwed on.

3. The Voltmaster potentiostat is connected to the 2 terminals of the CN ISE and set to measure an open circuit potential.

4. The electrode is allowed to reach equilibrium with the solution as evidenced by a constant solution potential reading. This represents the initial or baseline potential of the solution.

5. To initiate the reaction, 50 µL of a 21 mM solution of acetic anhydride (analyte) in acetone is injected into the cell, resulting in a final analyte concentration in solution of 50 µM.

6. Once the reaction has completed and the solution potential has once again stabilized, the final potential is recorded.

7. Before proceeding to the next experiment, the electrode is washed in DI water to remove any trace cyanide.
Beaker scale electrochemical studies (oxime initially in organic solvent)

The procedure for this section is nearly identical to that of the previous section, with the exception of steps #1-2.

1. Oxime is first dissolved in the organic solvent being tested in a 10 mg Oxime per 1 mL solvent ratio. This solution is then allowed to sit for a specified amount of time before being sampled for analysis.

2. The electrochemical cell is filled with 9 mL of blank buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH) and the Teflon® cap with the CN ISE is screwed on. At the designated analysis time, 1 mL of the oxime/organic solvent solution is added, and thoroughly mixed. This results in a 4.75 mM oxime solution in the electrochemical cell.

Microsensor performance studies (oxime directly in buffer)

All microsensor experiments followed the general procedures outlined below.

1. Oxime solution to be analyzed is first prepared and allowed to wait the specified amount of time. The 4.75 mM oxime solution is prepared by dissolving 1mg of oxime per 1 ml of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH). This solution is then allowed to sit for a specified amount of time before being sampled for analysis.

2. At the designated analysis time, this solution loaded into a syringe and slowly introduced into the liquid channel of the sensor.

3. The sensor, amp/filter and oxime containing syringe are all loaded into the GC oven set at 25°C. The sensor is then allowed to come to equilibrium for 20 – 30 minutes before any analyte is introduced.

4. A small amount of acetic anhydride is put in a GC vial to be used as the analyte during test. The GC automatic injector is then set to sample 1 µL of the headspace of the vial.
5. The GC is set at a 3000:1 split ratio to dilute the acetic anhydride vapor to 0.033% of its initial concentration in the helium carrier gas.

6. When an injection is started, the sampling needle automatically draws in ambient air 6 times to “wash” the syringe. The acetic anhydride vapor is then sampled, injected, diluted by the split flow, and set through the capillary to the sensor as a “pulse”. The electrochemical response of the sensor is then picked up by the external potentiostat. During the test, the oxime solution remains static to maximize the potential response.

7. Once the test has finished, the oven is opened, and fresh oxime solution is again slowly injected into the sensor to remove reaction products and reset the experiment.

Micro-sensor performance studies (oxime initially in organic solvent)

The procedure for this section is nearly identical to that of the previous section, with the exception of steps #1-2.

1. Oxime is first dissolved in the organic solvent being tested in a 10 mg Oxime per 1 mL solvent ratio. This solution is then allowed to sit for a specified amount of time before being sampled for analysis.

2. At the designated analysis time, 9 mL of buffer solution (25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH) is mixed with 1 mL of the oxime/organic solvent solution, and thoroughly mixed. This results in a 4.75 mM oxime solution for use in the micro sensor.

Micro-mixer performance studies

The micro-mixer comparison test was conducted following the steps below.

1. An organic solution of 11 mg oxime per 1 ml of ethanol is created fresh for the test. This solution is loaded into the 4.5 mm diameter syringe.
2. The 14 mm diameter syringe is loaded with 25 mM borate buffer solution, adjusted to pH 10 with concentrated NaOH.

3. Due to syringe pump limitations, the minimum reliable plunger speed is roughly 30 µm/min. This speed gives an aqueous buffer solution flow rate of 5.0 µL/min and an organic oxime solution flow rate of 0.5 µL/min.

4. A syringe containing acetic anhydride vapor dilute to 100 ppb using ambient air is loaded into the second syringe pump, which is set to provide a gas flow rate of 1 mL/min.

5. The open circuit potential of the sensor is monitored to determine when the system has reached a steady-state equilibrium. This potential corresponds to the initial or baseline reading.

6. The test is then started by initiating the flow of analyte vapor. This gas stream is flows through the sensor for 100 seconds and then turned off. The sensor is then allowed to return to its initial potential reading. The liquid syringe pump remains operational and pumping fluid at the designated flow rate during the entire test.

7. The comparison test is conducted by flushing out the system with DI water, and preparing fresh organic oxime and aqueous buffer solution as per step #1.

8. The aqueous buffer will be combined with the organic oxime solution in a 10:1 ratio again, however this time they will be mixed externally by hand.

9. This pre-mixed solution is then loaded into a syringe and the syringe pump is then set to deliver the fluid at a flow rate of 5.5 µL/min.

10. The rest of the comparison test is then carried out by following steps 4 – 6.