Inhibition-based biosensors for arsenic detection in water

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The findings and conclusions of this presentation have not been formally disseminated by U.S. EPA and should not be construed to represent any agency determination or policy.
The Problem As in ground water

Total Arsenic in untreated PWS ground water in Ohio

Arsenic concentrations (ug/L)
- 0.00 - 2.99
- 3.00 - 9.99
- > 10

Lithology
- Sand & Gravel Aquifers
- Interbedded Shale/Sandstone
- Sandstone Aquifers
- Carbonate Aquifers
- Interbedded Shale/Carbonate

Ohio Environmental Protection Agency
Division of Drinking and Ground Waters

USGS
Standard methods for detecting arsenic

• Lab-based instruments
  – Atomic Absorption Spectrometry.
  – Atomic Fluorescence Spectrometry.
  – ICP-MS.
  – Costly and require trained operators.

• Portable techniques
  – Stripping voltammetry.
  – Arsenic test kits.
  – More cost effective, but many contain toxic components. Issues with reliability at low concentrations.

https://en.wikipedia.org/wiki/
Project Goals

• Develop an arsenic test kits that has a reliable working range of 100 to 3000 µg/L (1.3 µM – 40 µM arsenic).
• Sensor has little or no interference with other ions drinking water.
• Sensor is not cumbersome and complicated.
• Develop a low cost, reliable, non-toxic and sensitive method for testing drinking water from wells.
  – Ideally with a detection limit <10 µg/L (133 nM).
Biosensor Applications

• Glucose biosensors
• Pregnancy test strips
• Alcohol breathalyzers
• Drugs and explosives
• Food toxins
• Pathogens
• YSI bioprocess analyzers
The Glucose Biosensor

- **Glucose Oxidase**
  - MW ~ 160 kDa
  - 280kDa subunits

\[
\text{GOx} + \text{glucose} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{gluconolactone} + \text{H}_2\text{O}_2
\]

From www.ysi.com
Inhibition Biosensor

- Monitor catalytic turnover of enzyme
- Inhibitors reduce turnover
- Commercially available CWA and Pesticide sensors

Agentase™ Chemical Agent Detection Kit

Agri-Screen® Tickets
Enzyme Inhibition Based Sensor for Arsenic Detection

• From literature there are many potential candidate enzymes that are reported to be inhibited by the various forms of arsenic.
  – Pyruvate dehydrogenase
  – Acetylcholinesterase
  – Acid Phosphatase
  – Urease
  – Cytochrome C
  – Others
    • Proteases, RNAases
    • Glutathione Reductase
  – Many potential sources, mammalian, plant, bacterial, etc.

• Inhibition appears to be primarily driven by modification of thiols (arsenite) or displacement of phosphate ions (arsenate).
Acetylcholinesterase (AChE)

- Acetylcholinesterase is well documented to be inhibited by As(III). A handful of groups have developed optical and amperometric biosensors using this enzyme.
- Closely related butyrylcholinesterase and many mutants of this enzyme are available.
- This enzyme is extremely sensitive to organophosphate and carbamate pesticides.
AChE Inhibition by As (III)

- Main As species As(III) and A(IV) – As(III) and order of magnitude more toxic
- Not sure of exact mechanism of inactivation.
  - Possible modification of tyrosine residue in active site (Wilson)
  - Literature suggest As(III) is a “quasi-irreversible inhibitor”
  - 2-pralidoxime can increase inhibition rate by >100x

Inhibition tests performed in 100 mM Sodium Phosphate, pH 7.4.
Remove aliquots at various time points and assay using Ellman’s method.
No inhibition by arsenic (V) observed.
Characterizing AChE Inhibition by Arsenite

\[
E + I \Leftrightarrow EI
\]

\[
\frac{d[E]}{dt} = k_f [E][I] - k_r [EI]
\]

\[
K = \frac{k_f}{k_r} = \frac{[EI]_e}{[E]_e[I]_e}
\]

\(E = \text{enzyme}\)

\(I = \text{interference}\)

<table>
<thead>
<tr>
<th>Cholinesterase</th>
<th>(K_i)</th>
<th>(k_f = 0.1322 \text{ min}^{-1}\text{mM}^{-1})</th>
<th>(k_r = 0.0035 \text{ min}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric Eel AChE</td>
<td>0.011 mM</td>
<td></td>
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<tr>
<td>Human AChE</td>
<td>0.15 mM</td>
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<td></td>
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<tr>
<td>Equine BChE</td>
<td>0.3 mM</td>
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</tbody>
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Electroactive AChE Substrates

Acetylthiocholine Chloride
(couple with Choline oxidase to Produce hydrogen peroxide)

Acetylthiocholine Chloride

p-Aminophenyl acetate

4-Acetoxy phenol

p-Nitrophenyl acetate
Electroactive AChE Substrates

- Acetylthiocholine Chloride
  (couple with Choline oxidase to produce hydrogen peroxide)

Acetylthiocholine Chloride

Acetylthiocholine Chloride
Electrochemical Detection of Thiocholine

\[ \text{Thiocholine} \xrightarrow{350 \text{ mV}} \text{Dithio-bis-choline} + 2\text{H}^+ + 2\text{e}^- \]

[Diagram showing electrochemical reaction and molecular structures]
Cyclic Voltammetry of Thiocholine Oxidation

Current (A) vs. Potential (V)

Thiocholine Oxidation:

-0.3 to 0.7 V

Thiocholine

Dithio-bischoline

2H\textsuperscript{+} + 2 e\textsuperscript{-}

cobalt Phthalocyanine
Amperometric Detection of Thiocholine

\[ y = 4.7103x - 0.056 \]
\[ R^2 = 0.9957 \]
Immobilization of AChE on Electrode

- Covalent Crosslinking using Glutaraldehyde.
  - Adsorb AChE with Bovine Serum Albumin (BSA) to electrode surface.
  - Crosslink using glutaraldehyde.

Adsorb AChE with Bovine Serum Albumin to Electrode Surface

Crosslink Proteins Using Glutaraldehyde
Immobilization of AChE on Electrode

- Enzyme activity on electrode is stable and does not wash-out after repeated testing.
Immobilization of AChE on Electrode

- Enzyme immobilized on electrode follows Michaelis-Menten kinetics.
- $K_m$ increases with decreasing BSA.
- “Maximum” current decreases with decreasing BSA.
Electrode Inhibition by Arsenite

- Inhibition tests were performed in 25 mM Tris-HCl, pH 7.
- Electrodes tested for AChE activity using 1 mM acetylthiocholine.
- Degree of inhibition is dependent on incubation time.
Developing a More Sensitive Inhibition Biosensor

- The inhibition constant, $K_i$, for the enzyme with arsenite will determine the maximum detection limit of the inhibition biosensor.
- Development of a biosensor with an enzyme with a lower $K_i$ should improve the detection limit.
Conclusions

- Acetylcholinesterase from electric eel is inhibited by arsenic (III) with an inhibition constant of 11 µM. The enzyme is no inhibited by arsenic (V).

- Acetylcholinesterase was immobilized on a screen printed electrode using glutaraldehyde and bovine serum albumin. The immobilized enzyme followed Michaelis-Menten kinetics.

- The acetylcholinesterase biosensor was inhibited by arsenic (III). The working range of the biosensor was between 10 µM and 1000 µM.

- Development of a biosensor using an enzyme with a smaller inhibition constant should lead to a lower detection limit.
Thank you!
Acknowledgements

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