A disposable acetylcholine esterase sensor for As(III) determination in groundwater matrix based on 4-acetoxyphenol hydrolysis

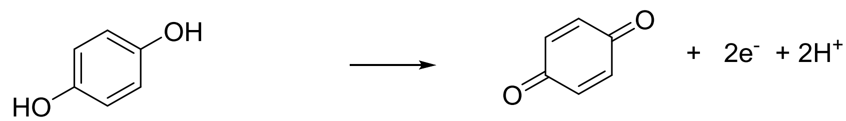
Tao Li[1]*, Endalkachew Sahle-Demessie[1], Jason Berberich[2], Eunice Varughese[1]

[1]National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio, 45268, USA

[2]Department of Chemical, Paper and Biomedical Engineering, Miami University, Oxford, Ohio, 45056, USA

Supporting Information

1. Cyclic voltammetry study for hydroquinone



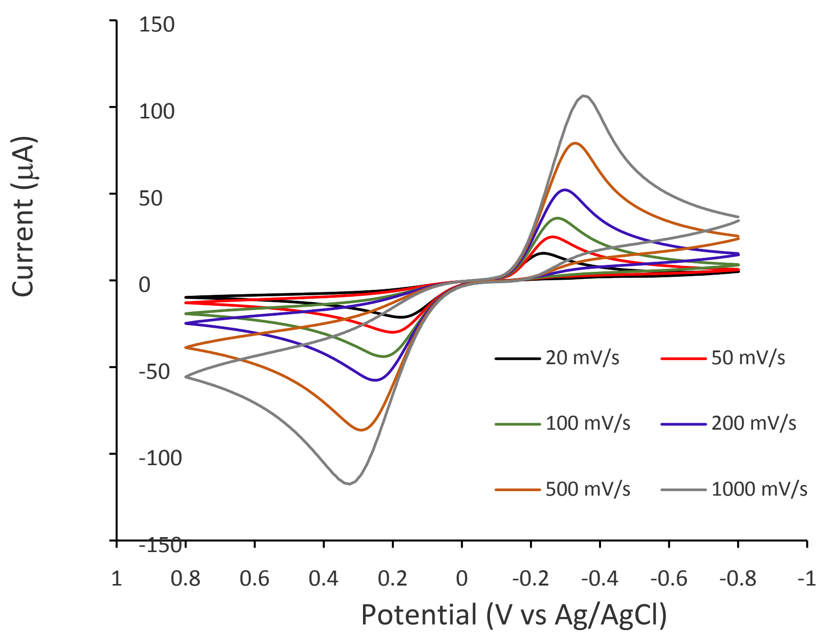


Figure 1. Cyclic voltammograms for hydroquinone (5 mM in 0.1 M phosphate, pH 7.0, 23 °C)

Table 1. The impact of scan rate on the voltammograms of hydroquinone

Scan Rate (mV s⁻¹)	E_{pa} (V)	i_{pa} (µA)	E_{pc} (V)	i_{pc} (µA)	i_{pa}/i_{pc} (µA)	ΔE (V)
20	0.181	22.499	-0.239	20.592	1.09	0.420
50	0.207	30.076	-0.259	31.684	0.95	0.466
100	0.225	36.859	-0.282	43.259	0.85	0.507
200	0.245	53.975	-0.302	55.109	0.98	0.547
500	0.286	78.258	-0.330	77.501	1.01	0.616
1000	0.319	103.430	-0.353	104.971	0.99	0.672

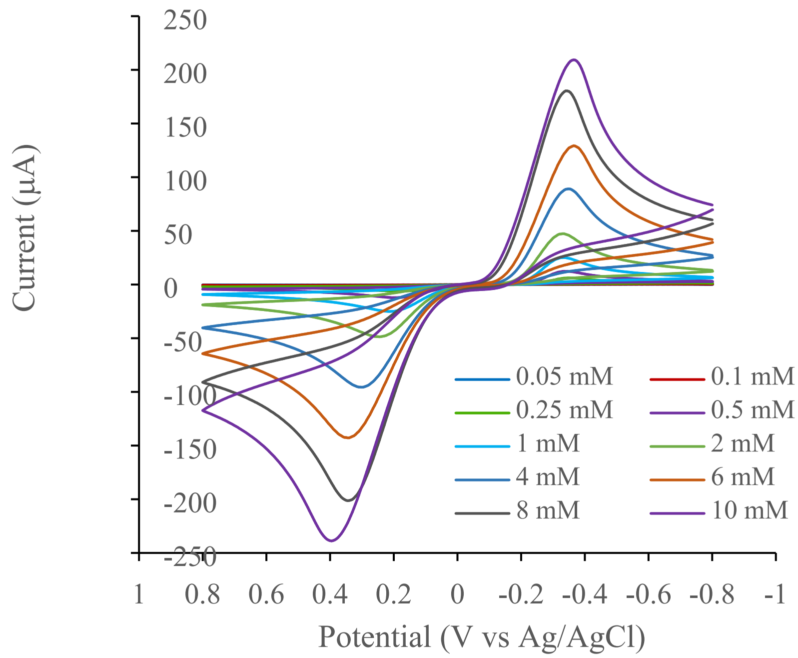


Figure 2. Cyclic voltammetry of H_2Q at 1000 mV/s in 0.1 M phosphate, pH 7.0

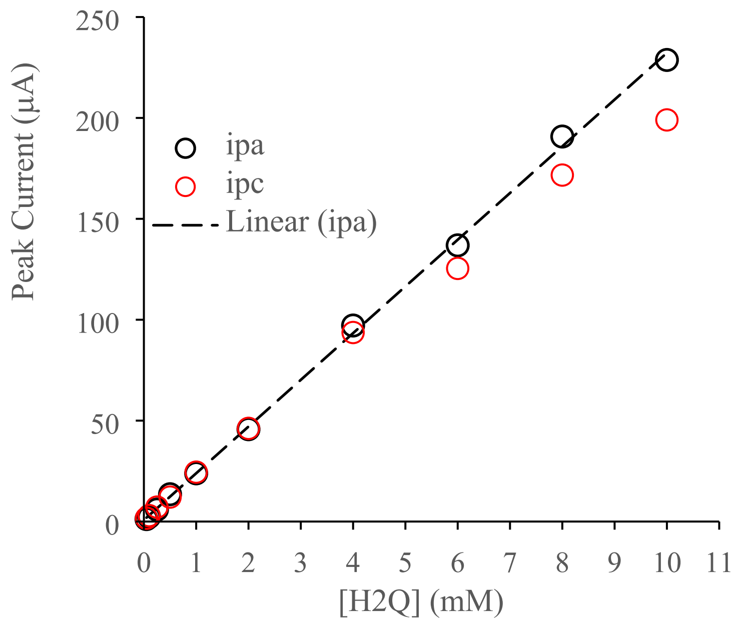


Figure 3. Standard curve of peak current of H_2Q with CV at 1000 mV/s scan rate

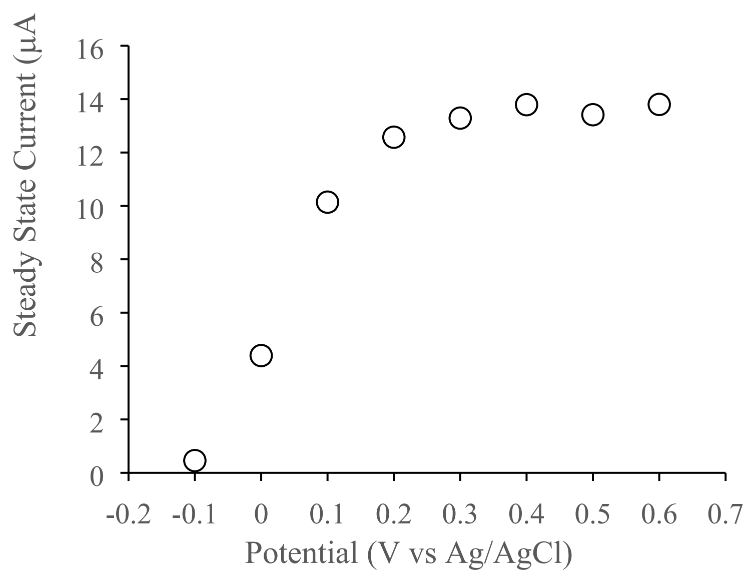


Figure 4 Voltammogram of hydroquinone (2 mM in 0.1 M phosphate, pH 7.0)

Table 2. Impact of crosslinking factors on the steady state current and inhibition by 1 mM As(III) (The concentration of AchE was fixed at 5.3 μg/electrode)

Electrode	pH	BSA (μg)	GA (%)	Steady State Current (μA)	Inhibition (%)
1	7.5	30	0.015	82.2	59.1
2	7	20	0.015	87.5	65.7
3	6.5	30	0.005	77.4	69.6
4	7	20	0.01	79.2	61.7
5	7	10	0.01	88.6	63.0
6	6.5	20	0.01	90.9	68.0
7	7.5	20	0.01	81.2	63.4
8	6.5	30	0.015	86.2	65.1
9	7.5	10	0.015	65.9	67.1
10	6.5	10	0.005	93.3	71.7
11	7	30	0.01	105.3	65.8
12*	7.5	30	0.005	36.3	74.7
13	7.5	10	0.005	106.1	70.7
14	7	20	0.01	88.0	66.9
15	6.5	10	0.015	70.0	66.0
16	7	20	0.005	95.2	69.3

*Electrode 12 was excluded as an outlier as the steady sate current was atypically low.

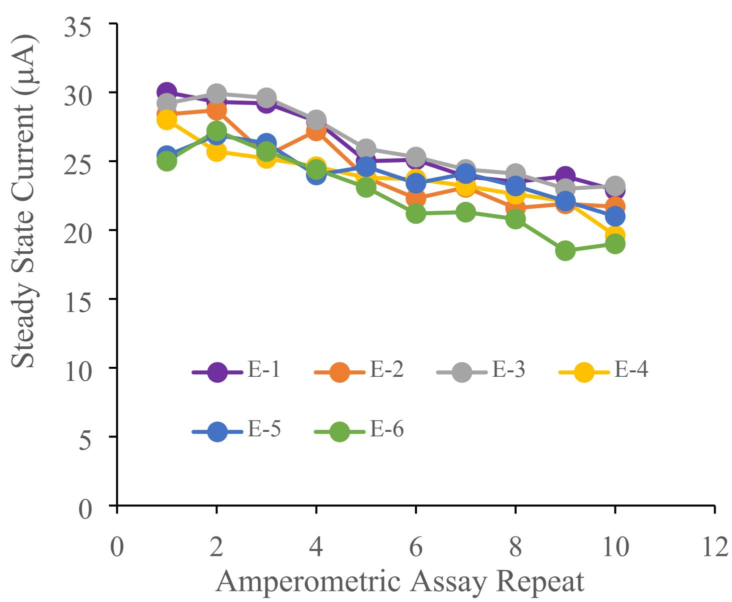


Figure 5. Change of steady state current of the AchE electrode in repeated uses

Table 3. The composition of a synthetic groundwater based on the water chemistry in Shepley's Hill Landfill in Fort Devens, MA.

Salt	[cation] (μM)	CO_3^{2-}(μM)	Cl^-(μM)	SO_4^{2-}(μM)	Silicate(μM)	PO_4^{3-} (μM)
$FeCl_2$	612		612			
$MnCl_2$	83		83			
$CaCO_3$	1105	1105				
K_2CO_3	213	106				
$MgSO_4$	256			256		
$NaHCO_3$	847	847				
$(NH_4)_2CO_3$	143	72				
Na_2SiO_3	400				400	
Na_2HPO_4	100					100
Total		2130	695			

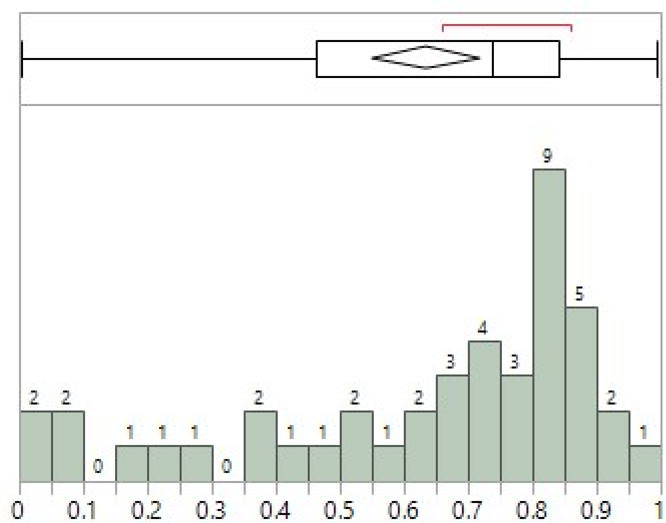


Figure 6. Distribution of total As (mg L^{-1}) in 43 groundwater samples from Shepley's Hill Landfill Superfund Site at Fort Devens, MA. The samples were taken between May 18, 2006 and October 30, 2007.

Modeling AchE inactivation by As (III)

1. **Reaction**

$$\text{AchE} + \text{As(III)} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{AchE As(III)}$$

Eq 1

Assumption: [As] >> [AchE], therefore this reaction can be reduced to a reversible pseudo-first order reaction.

$$\text{E} \underset{k_{-1}}{\overset{k_1[\text{As}]}{\rightleftharpoons}} \text{EAs}$$

Eq 2

The unit for constant: k_1: M^{-1} min^{-1}, k_{-1}: min^{-1}.

2. **Rate law and kinetic model**

$$-\frac{d[E]}{dt} = k_1[As][E] - k_{-1}[EAs]$$

Eq 3

Assumption: when reaching the equilibrium, the concentration of free enzyme is E_∞

Mass balance relationship: $E_0 = E + EAs = E_\infty + EAs_\infty$, **Eq 4**

At equilibrium: $$-\frac{d[E]}{dt} = 0 = k_1[As][E]_\infty - k_{-1}[EAs]_\infty$$

Or: $$[EAs]_\infty = \frac{k_1}{k_{-1}}[As][E]_\infty$$

Eq 5

Substitute $[EAs]_\infty$ in **Eq 4** and rearrange the equation to relate [E] and [EAs]

$$[EAs] = [E]_\infty + \frac{k_1}{k_{-1}}[As][E]_\infty - [E]$$

Eq 6

Substitute **Eq 6** into rate law **Eq 3**.

$$-\frac{d[E]}{dt} = k_1[As][E] - k_{-1}[E]_\infty - k_1[As][E]_\infty + k_{-1}[E]$$

Eq 7

Factor **Eq 7** for integration

$$-\frac{d[E]}{dt} = (k_1[As] + k_{-1})([E] - [E]_\infty)$$ **Eq 8**

Integration of **Eq 8** gives kinetic model

$$\ln\frac{[E] - [E]_\infty}{[E]_0 - [E]_\infty} = -\left(k_1[As] + k_{-1}\right)t$$ **Eq 9**

3. The relationship between E_∞ and E_0

Based on the two equations, solve for $\frac{E_\infty}{E_0}$

$$E_0 = E_\infty + EAs_\infty,$$ **Eq 4**

$$[EAs]_\infty = \frac{k_1}{k_{-1}}[As][E]_\infty$$ **Eq 5**

$$\frac{E_\infty}{E_0} = \frac{k_{-1}}{k_{-1} + k_1[As]}$$ **Eq 10**

4. Determination of k_1 and k_{-1} through least-square fitting

Independent variables: t (s^{-1}), and [As]

Dependent variable: $\hat{y} = \frac{[E]}{[E]_0}$

Parameters (coefficients): k_1 and k_{-1}.

The relationship between dependent variable $\frac{[E]}{[E]_0}$ and $\ln\frac{[E] - [E]_\infty}{[E]_0 - [E]_\infty}$ in the model (**Eq 9**)

Let $\frac{[E] - [E]_\infty}{[E]_0 - [E]_\infty} = a$, rearrange gives $\frac{E}{E_0} = a + (1 - a)\frac{E_\infty}{E_0}$ **Eq 11**

From **Eq 9**:

$$a = e^{-\left(k_1[As] + k_{-1}\right)t}$$ **Eq 12**

Estimation of $\hat{y}$:

- Select starting value for k_1 and k_{-1}
- Determine a with **Eq 12**
- Determine $\frac{E_\infty}{E_0}$ with **Eq 10**
- Estimate dependent variable $\hat{y}$ with **Eq 11**

Determine Sum of Square Residues (SSR): $SSR = \sum(\hat{y} - y)^2$

Fitting to estimate k_1 and k_{-1} by minimizing SSR