Lecture 3

Potential-Controlled Techniques in Electrochemical Sensing.
Enzymatic Electrodes.
Cyclic voltammetry

- The most widely used technique for acquiring **quantitative** information about e/chemical reaction
- Involves linear scanning of the working electrode potential

For reversible reaction:

\[ E^0 \approx E \pm \frac{90}{z} \text{ mV} \]
Cyclic voltammetry

• initial situation

• formal potential reached in forward scan
  \[ E = E^0 + RT \ln \left( \frac{Ox}{Re} \right) \]

• max. current

• formal potential reached in reversed scan
Cyclic voltammetry

- The peak current for a reversible system is given by Randles-Sevcik equation:

\[ i_p = \left(2.69 \times 10^5\right) n^{3/2} ACD^{1/2} v^{1/2} \]

A in cm\(^2\), C in mol/cm\(^3\), D cm\(^2\)/s, v in V/s, at 25°C.

- The formal potential for a reversible couple:

\[ E^0 = \frac{E_{p,a} + E_{p,c}}{2} \]

- The separation between the peaks gives information on the number of electrons transferred (for a reversible couple)

\[ \Delta E_p = E_{p,a} + E_{p,c} = \frac{59mV}{z} \]
Cyclic Voltammetry

• For irreversible processes (where electron transfer is a limiting factor) peak potential will depend on the scan rate

\[ E_p = E^\circ - \frac{RT}{\alpha n F} \left[ 0.78 - \ln \frac{k^\circ}{D^{1/2}} + \ln \left( \frac{\alpha n F v}{RT} \right)^{1/2} \right] \]

\[ i_p = (2.99 \times 10^3)n(\alpha n_a)^{1/2} ACD^{1/2}v^{1/2} \]

where \( \alpha \) is electron transfer coefficient

at \( \alpha = 0.5 \) peak height drops to 80%
Cyclic voltammetry

• Cyclic voltammetry in the presence of competing chemical reaction can be used to study kinetics

\[ O + ne^- \rightleftharpoons R \rightarrow Z \]

• Example: electro reduction of p-bromonitrobenzene

\[ \text{BrC}_6\text{H}_4\text{NO}_2 + e^- \rightarrow \text{BrC}_6\text{H}_4\text{NO}_2^- \]
\[ \text{BrC}_6\text{H}_4\text{NO}_2^- \rightarrow \bullet \text{C}_6\text{H}_4\text{NO}_2 + \text{Br}^- \]
\[ \bullet \text{C}_6\text{H}_4\text{NO}_2^- + e^- \rightarrow \text{C}_6\text{H}_4\text{NO}_2^- \]
\[ \text{C}_6\text{H}_4\text{NO}_2^- + H^+ \rightarrow \text{C}_6\text{H}_5\text{NO}_2 \]
Cyclic voltammetry

- In the case of adsorption process on the electrode, the separation between the peaks will be smaller and current will be proportional to the adsorption

\[ i_p = \frac{n^2 F^2 \Gamma A v}{4RT} \]

\[ Q = nFA \Gamma \]
Spectroelectrochemistry

- Optical techniques, e.g. spectroscopic adsorption can be coupled to e/chemical methods

optically transparent electrode (e.g. metal grid, thin metal layer, ITO etc.)
Chronoamperometry

- involves stepping potential of the working electrode from a value when no faradaic current occurs to a potential at which the concentration of electactive species becomes zero
- Response described by Cottrell equation:

\[ i(t) = \frac{nFAD_oC_0(b)}{\sqrt{\pi D_0t}} = kt^{-1/2} \]

- Anson plot:

\[ Q(t^{1/2}) \]

\[ Q(t) = \frac{nFAC_0(b)\sqrt{D_0t}C_0(b)}{\sqrt{\pi}} + Q_{dl} + Q_t \]

- first 50ms – contribution of charging current
- longer than 100s – contribution of convection effects, electrode non-planarity, reactions etc.
Polarography

- subclass of voltammetry when dropping mercure electrode (DME) is used as a working electrode
- due to the impact of the technique on the electroanalysis its inventor J. Heyrovsky was awarded a 1959 Nobel price in Chemistry
Pulse voltammetry techniques are aimed at lowering the detection limits (down to $10^{-8}$ M!) by reducing the ratio between faradaic and non-faradaic currents.

The difference between the different pulse techniques:
- excitation waveform
- sampling of current
Normal-Pulse Voltammetry

- consists of series of pulses with increasing amplitude (in case of DME applied to successive drops near the end of the drop lifetime).

\[ i(t) = \frac{nFAD_0C_0(b)}{\sqrt{\pi D_0 t_m}} \]

- Advantages:
  - due to short pulse duration, the diffusion layer is thinner and therefore higher faradaic current
  - almost zero charging current
Differential Pulse Voltammetry

- fixed magnitude pulses are superimposed on the linear potential ramp
- current sampled twice: before the pulse (1) and 40ms after the pulse begins

\[ \Delta i = t(t_2) - t(t_1) \text{ vs. } V \]

\[ E_p = E_{1/2} - \Delta E/2 \]

\[
i_p(t) = \frac{nFAD_O C_O(b)}{\sqrt{\pi D_O t_m}} \left(1 - \frac{\sigma}{1 + \sigma}\right) \]

\[
\sigma = \exp\left[\left(\frac{nf}{RT}\right)/(\Delta E/2)\right]
\]
Differential Pulse Voltammetry

- allows measurement down to 10-8 M concentration
- improved resolution between the species with similar potential (down to 50 mV)
- typical parameters:
  - pulse 25-50 mV
  - scan rate 5mV/s

mixture of Cd$^{2+}$ and Pb$^{2+}$ in 0.1M HNO$_3$. 
Square-Wave Voltammetry

- large-amplitude differential technique, the reverse pulse causes the reverse reaction of the product
- the current is sampled twice: at the end of the forward pulse and at the end of the reversed pulse
Square-Wave Voltammetry

- major advantage – speed, complete voltammogram can be recorded within a couple of seconds
- advantageous in batch and flow analytical operations, can resolve neighboring peaks in chromatography and capillary electrophoresis
Staircase Voltammetry

- Voltage is increased in steps of ~10 mV with 50 ms delay.
- Response similar to cyclic voltammetry but with reduced charging current.
AC Voltammetry

- small amplitude of AC is superimposed on linear ramp
- for a reversible system the response is similar to derivative of the DC response
- detection of AC components allows separation of faradaic current (45° with excitation) and charging (90° with excitation)
- detection limit ~5×10⁻⁷ M
- large amplitude AC (>50mV) allows identification of specific components via higher harmonics “fingerprinting”
- the height of the peak is proportional to the concentration, amplitude and sq.root of frequency

\[ i_p = \frac{n^2 F^2 A \omega^{1/2} D^{1/3} C \Delta E}{4RT} \]
Stripping analysis

• the idea:
  – first pre-concentrate the analyte on the surface of the electrode
  – then strip (dissolve) the analyte and measure
• detection levels down to $10^{-10}$ M is feasible
• varios variations exists:
  – anodic stripping voltammetry
  – potentiometric
  – adsorptive stripping
  – cathodic stripping
  – abrasive stripping
Anodic Stripping Voltammetry

- Pre-concentration is done by amalgaming the metal in question in small volume mercury electrode

\[ M^{n+} + ne^- + Hg \rightarrow M(Hg) \]

- The concentration can be calculated from the pre-concentration current measured

\[ C_{Hg} = \frac{i_1 t_d}{n F v_{Hg}} \]

- During the anodic scan the metal is re-oxidated and stripped from the electrode

\[ M(Hg) \rightarrow M^{n+} + ne^- + Hg \]
Anodic Stripping Voltammetry

- potential scan
- voltammogram
Potentiometric Stripping Analysis

• the oxidation step is done using an oxidation agent (O2, Hg(II) etc.) present in the solution

\[ M(Hg) + \text{oxidant} \rightarrow M^{n+} \]

• potential of the electrode is measured vs time
Adsorptive Stripping Voltametry

• pre-concentration goes via adsorption of a metal ion in a surface bound complex (instead of amalgaming)

• Langmuir kinetics of adsorption vs time

• extremely low detection limits can be achieved (down to $10^{-12}$ M)
Cathodic stripping voltammetry

- involves anodic deposition of analyte followed by negative-going potential scan for detection of anions in the solution

\[ A^{n-} + Hg \xleftarrow{\text{deposition}} \xrightarrow{\text{stripping}} HgA + ne^{-} \]

- suitable for a wide range of compounds forming insoluble salts with mercury (halide ions, thiols, penicillins etc.)
- silver and copper can be used in a similar manner
• mechanical (abrasive) transfer of solid material onto an electrode surface (e.g. paraffin coated graphite)
Flow analysis

- Electrochemical techniques can be combined with chromatography (flow) analysis to identify the components present.

Capillary electrophoresis/amperometric analysis of Bud Light beer.
Flow analysis

• thin layer cell design

• thin layer cell design
Amperometric Biosensors with Enzyme Electrodes

- First Generation – oxygen electrode based sensors
- Second Generation – mediator based sensors
- Third Generation – directly coupled enzyme electrodes
Possible glucose detection schemes

1st generation schemes

2nd generation schemes

3rd generation schemes

Gluconic acid → pH

GOD_{Ox}

GOD_{R}

E ↔ O_2

E ↔ H_2O_2

PO → 2H^+

2H^- ↔ E

2 H_2O + 1/2 O_2
Clark-type glucose electrode

\[
\text{glucose} + \text{O}_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + \text{H}_2\text{O}_2
\]
Reactions at the Oxygen electrode (1st generation)

- Reaction at the enzyme electrode

\[
\text{glucose} + O_2 \xrightarrow{GOX} \text{gluconic acid} + H_2O_2
\]

Measuring oxygen:

\[
O_2 + e^- \rightarrow O_2^- \quad E=-0.7V
\]

Problems: fairly high potential (interference is probable), oxygen needs to be controlled and replenished (e.g. by oxygen generating reaction, by pumping oxygen containing buffer, re-cycling H_2O_2 using catalaize etc. )

Measuring hydrogen peroxide:

\[
H_2O_2 \rightarrow 2H^+ + 2e^- + O_2 \quad E=+0.65V
\]

Problem: still fairly high potential (interference from e.g. ascorbic asid)
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Enzyme</th>
<th>Response time (min)</th>
<th>Stability (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Glucose oxidase</td>
<td>2</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol oxidase</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Monoamines</td>
<td>Monoamine oxidase</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Oxalate</td>
<td>Oxalate oxidase</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>Lactate</td>
<td>Lactate oxidase</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Aldehyde oxidase</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Alcohol oxidase</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glycollate</td>
<td>Glycollate oxidase</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NADH</td>
<td>NADH oxidase</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Mediator Based Sensors

- Oxygen is substituted with another oxidizing agent (electron transfer agent)
- Iron ions or complexes are most common mediators
Free $\text{Fe}^{3+}$ are subject to hydrolysis and precipitation.
glucose + GOD_{Ox} \rightarrow \text{gluconolactone} + GOD_{R} + 2H^{+} \\
GOD_{R} + 2Fc^{+} \rightarrow GOD_{Ox} + 2Fc \\
2Fc - 2e^{-} \rightarrow 2Fc^{+}
Good Mediator

- Rapid reaction with enzyme
- Fast electron transfer kinetics
- Low overpotential
- Independent of pH
- Stable in Ox and R forms
- Doesn’t react with oxygen
- Non toxic
Fc derivatives

Rate constant for electron transfer to the enzyme

<table>
<thead>
<tr>
<th>Derivative</th>
<th>$E$ (V)$^a$</th>
<th>$k$ (10$^5$ dm$^3$ mol$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1'-Dimethyl</td>
<td>0.100</td>
<td>0.8</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.142</td>
<td>—</td>
</tr>
<tr>
<td>Ferrocene$^b$</td>
<td>0.165</td>
<td>0.3</td>
</tr>
<tr>
<td>Amidopentylaminidoppyrrole</td>
<td>0.200</td>
<td>2.07</td>
</tr>
<tr>
<td>Aminopropylpyrrole</td>
<td>0.215</td>
<td>0.75</td>
</tr>
<tr>
<td>Vinyl</td>
<td>0.253</td>
<td>0.3</td>
</tr>
<tr>
<td>Monocarboxylic acid</td>
<td>0.275</td>
<td>2.0</td>
</tr>
<tr>
<td>1,1'-Diacarboxylic acid</td>
<td>0.290</td>
<td>0.3</td>
</tr>
<tr>
<td>Methyltrimethylamino</td>
<td>0.387</td>
<td>5.3</td>
</tr>
<tr>
<td>Polyvinyl</td>
<td>0.435</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$Versus the saturated-calomel electrode (SCE).

$^b$Deposited from ferrocene powder.

Compare with -0.7V required for Clarks electrode.
Various mediators (natural and artificial)

<table>
<thead>
<tr>
<th>Natural</th>
<th>$E (V)^a$</th>
<th>Artificial</th>
<th>$E (V)^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome $a_3$</td>
<td>+0.29</td>
<td>Hexacyanoferrate(III)</td>
<td>+0.45</td>
</tr>
<tr>
<td>Cytochrome $c_3$</td>
<td>+0.24</td>
<td>2,6-Dichlorophenol</td>
<td>+0.24</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>+0.10</td>
<td>Indophenol</td>
<td>+0.24</td>
</tr>
<tr>
<td>Cytochrome $b$</td>
<td>+0.08</td>
<td>Ferrocene</td>
<td>+0.17</td>
</tr>
<tr>
<td>Vitamin $K_2$</td>
<td>−0.03</td>
<td>Phenazine methosulphate</td>
<td>+0.07</td>
</tr>
<tr>
<td>Rubredoxin</td>
<td>−0.05</td>
<td>Methylene Blue</td>
<td>+0.04</td>
</tr>
<tr>
<td>Flavoproteins</td>
<td>−0.4 to +0.2</td>
<td>Phthalocyanine</td>
<td>−0.02</td>
</tr>
<tr>
<td>FAD/FADH$_2$</td>
<td>−0.23</td>
<td>Phenosafranine</td>
<td>−0.23</td>
</tr>
<tr>
<td>FMN/FMNH$_3$</td>
<td>−0.23</td>
<td>Benzylviologen</td>
<td>−0.36</td>
</tr>
<tr>
<td>NAD$^+$/NADH</td>
<td>−0.32</td>
<td>Methylviologen</td>
<td>−0.46</td>
</tr>
<tr>
<td>NADP$^+$/NADPH</td>
<td>−0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferridoxin</td>
<td>−0.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Versus the standard hydrogen electrode (SHE).
How it works...

In real biosensors both GOD and Fc are immobilised

\[ \text{Fc} + \text{glucose} + \text{GOD} \]

\[ \text{Fc} + \text{glucose} \]
Generally, the enzyme might denature on the electrode surface;

- electron transfer reaction might be slow
- Thus, the surface has to be modified...

- Enzymes can be directly wired to the electrode using organic conducting salts (e.g. TTF/TCNQ) or redox polymers
- Enzymes can be modified to facilitate electron transfer and attachment
Possible glucose detection schemes
Design example: Glucose sensor

- **Aim**: for use by patient at home (should be simple, reliable and cheap)
- **Performance**: blood glucose range 1.1-33.3 mM; precision 3-8%; test time 30s; life time 6 month.
- **Selective element**: Glucose Oxidase – inexpensive, stable over long period
- **Transducer**: Amperometric (GOD+Fc) – cheap, reliable, easy read-out with LCD.
- **Immobilisation**: covalent bonding for long life (graphite foil coated with Fc, GOD immobilised)
ExacTech Glucose Sensor

Diagram showing the components of the sensor:
- PVC substrate
- Contacts
- Conductive silver track
- Conductive carbon track
- Dielectric layer
- Ag–AgCl reference electrode
- Working electrode