Potentiometric Biochemical Sensors

Outline

Potentiometric transducing principle

Selectivity of potentiometric membrane electrodes

pH glass electrodes

Liquid membrane electrodes

Gas sensors

Enzyme electrodes
Potentiometric Transducing Principle

- Zero-current cell potential
- Fast response time
- Non-perturbing measurement device (the analyte is not consumed)
- Measuring ion activities rather than total concentrations
Zero-current cell potential

- The potential across the ion-selective membrane will be directly dependent on the ion composition of the sample and the inner electrolyte.

- All other potential contributions must be constant.

\[
E_{\text{CELL}} = (\phi_E - \phi_D) + (\phi_D - \phi_C) + (\phi_C - \phi_B) + (\phi_B - \phi_A)
\]

\[
E_{\text{CELL}} = E_4 + E_3 + E_2 + E_1
\]

\[E_3\] is typically constant if the ionic composition of the inner solution does not change.

\[
E_{\text{CELL}} = k + E_2
\]
Zero-current cell potential

- Sample ions can rapidly exchange with ion-selective membranes: 
  - interfacial equilibrium -

- What determines the mass transfer?
  Standard chemical potential differences 
  + free ion activity in each phase

Boundary potential counterbalancing the chemical tendency of ions to distribute
Zero-current cell potential

Ion in the aqueous phase

\[ \bar{\mu}_i(aq) = \mu_i(aq) + zF\phi(aq) \]

\[ = \mu_i^0(aq) + RT \ln[a_i(aq)] + zF\phi(aq) \]

Ion in the membrane

\[ \bar{\mu}_i(mem) = \mu_i^0(mem) + RT \ln[a_i(mem)] + zF\phi(mem) \]

In equilibrium

\[ \bar{\mu}_i(aq) = \bar{\mu}_i(mem) \]

\[ \Delta\phi \equiv E_{PB} \equiv E_2 = -\frac{\mu_i^0(mem) - \mu_i^0(aq)}{zF} + \frac{RT}{zF} \ln\frac{a_i(aq)}{a_i(mem)} \]

\[ k_i = \exp\left(\frac{\mu_i^0(aq) - \mu_i^0(mem)}{RT}\right) \]

\[ E_{PB} = \frac{RT}{zF} \ln k_i a_i(aq) \]

\[ \frac{RT}{a_i(mem)} \]
Zero-current cell potential

If the composition of the membrane is not altered

\[ E_{\text{CELL}} = E^0_I + \frac{RT}{zF} \ln a_I(aq) \]

Nernst Equation

\[ E_{\text{CELL}}(\text{mV}) = E^0_I + \frac{59.2}{z} \log a_I(aq) \]
Selectivity of potentiometric membrane electrodes

Competitive distribution results in interferences by other ions

Potentiometric selectivity coefficient $K_{IJ}^{pot}$ for an interfering ion $J$ with the specific ion $I$

$$K_{IJ}^{pot} = \exp\left(\frac{E_J^0 - E_I^0}{RT} z_I F\right)$$

$K_{IJ}^{pot}$ can be used to assess the extent of interference in a mixed sample...

$$E_{CELL} = E_I^0 + \frac{RT}{z_I F} \ln\left[a_I + \sum K_{IJ}^{pot} a_J^{z_I/z_J}\right]$$

Nicolsky-Eisenman equation

Optimization of ISE membranes by using very low $K_{IJ}^{pot}$
Selectivity of potentiometric membrane electrodes

It is also possible to work with non ideal selectivities if the primary ion is relatively concentrated

\[ K_{IJ}^{\text{pot}} \approx \frac{a_I}{(100a_J)^{z_I/z_J}} \]

\( \equiv \) required for interference < 1%

**Example:** 1mM NaCl, KCl, CaCl₂

i) Na⁺ ISE

\[ K_{Na,K}^{\text{pot}} < 0.01, \; K_{Na,Ca}^{\text{pot}} < 0.0031 \]

ii) Ca⁺⁺ ISE

\[ K_{Ca,Na}^{\text{pot}} = K_{Ca,K}^{\text{pot}} < 0.1 \]

reducing the concentration to 0.1mM

i) Na⁺ ISE

\[ K_{Na,K}^{\text{pot}} < 0.01, \; K_{Na,Ca}^{\text{pot}} < 0.001 \]

ii) Ca⁺⁺ ISE

\[ K_{Ca,Na}^{\text{pot}} = K_{Ca,K}^{\text{pot}} < 1 \]

---

Some common cations in blood

<table>
<thead>
<tr>
<th>Ion</th>
<th>( a_I(\text{min}) )</th>
<th>( a_I(\text{max}) )</th>
<th>( \text{Log}<em>{K</em>{IJ}^{\text{pot}}} ) (required) in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Interferent ( J )</td>
</tr>
<tr>
<td>Na⁺</td>
<td>( 1.02 \times 10^{-1} )</td>
<td>( 1.12 \times 10^{-1} )</td>
<td>( K^+ )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{Mg}^{2+} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{Ca}^{2+} )</td>
</tr>
<tr>
<td>K⁺</td>
<td>( 2.60 \times 10^{-3} )</td>
<td>( 3.70 \times 10^{-3} )</td>
<td>( \text{Na}^+ )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{Mg}^{2+} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{Ca}^{2+} )</td>
</tr>
<tr>
<td>( \text{Mg}^{2+} )</td>
<td>( 1.60 \times 10^{-4} )</td>
<td>( 2.80 \times 10^{-4} )</td>
<td>( \text{Na}^+ )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( K^+ )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{Ca}^{2+} )</td>
</tr>
<tr>
<td>( \text{Ca}^{2+} )</td>
<td>( 3.40 \times 10^{-4} )</td>
<td>( 4.10 \times 10^{-4} )</td>
<td>( \text{Na}^+ )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( K^+ )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{Mg}^{2+} )</td>
</tr>
</tbody>
</table>
**pH – Glass electrodes**

Typical glass composition:
72.2% SiO₂, 6.4% CaO, 21.4% Na₂O

Different composition required for selectivity to different ions

- Glass with high concentration of Na⁺ are sensitive to Na⁺

  Na⁺ activities in biological samples (at physiological pH, the activity of H⁺ is low compared to Na⁺ levels, 140mM in blood)

**Limitations**

- tendency to be fouled by strongly adsorbing proteins
- no sensitivity to anions
- fragile membranes…microfabrication?
Liquid membrane electrodes

Based on polymer membranes activated with special membranes components.

i) freely dissolved in this hydrophobic matrix.
ii) covalently anchored onto the polymeric backbone of the membrane

Main function of membrane components

a) selective extraction of analytes
b) Introduce ion-exchanger properties to the membrane

fixing $a_I^{(mem)}$

Preconditioning of the membrane

$ClO_4^-$, $NO_3^-$, $Cl^-$, $Br^-$, $I^-$, $CO_3^{2-}$
$Cs^+$, $K^+$, $Na^+$, $Li^+$
Gas sensors

Equilibration of gases in aqueous solution yield ionic species

<table>
<thead>
<tr>
<th>Analyte gas</th>
<th>Equilibrium reactions</th>
<th>Inner ion electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>CO₂ + H₂O ↔ H⁺ + HCO₃⁻</td>
<td>H⁺, CO₃²⁻</td>
</tr>
<tr>
<td>SO₂</td>
<td>SO₂ + H₂O ↔ H⁺ + HSO₃⁻</td>
<td>H⁺, HSO₃⁻, SO₃²⁻</td>
</tr>
<tr>
<td>NO₂</td>
<td>NO₂ + H₂O ↔ NO₃⁻ + NO₂⁻ + H⁺</td>
<td>H⁺, NO₂⁻, NO₃⁻</td>
</tr>
<tr>
<td>HOAc (acetic acid)</td>
<td>HOAc ↔ H⁺ + OAc⁻</td>
<td>H⁺, OAc⁻</td>
</tr>
<tr>
<td>NH₃</td>
<td>NH₃ + H₂O ↔ NH₄⁺ + OH⁻</td>
<td>H⁺, NH₄⁺</td>
</tr>
</tbody>
</table>

Combination of ISE-based cells as detectors behind outer gas permeable membranes

\[ E_{\text{CELL}} = k + 0.059 \log a_{H^+} \]

\[ K_{CO_2} = \frac{a_{H^+} \times a_{HCO_3^-}}{P_{CO_2}} \]

\[ E_{\text{CELL}} = k + 0.059 \log \frac{K_{CO_2} P_{CO_2}}{a_{HCO_3^-}} \]

Using a fairly concentrated NaHCO₃ internal filling solution (0.1M)

\[ E_{\text{CELL}} = k' + 0.059 \log P_{CO_2} \]
Gas sensors

\[ \text{NH}_3 \text{ sensor} \quad \text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^- \]

and using \( \text{NH}_4\text{Cl} \) solutions as inner electrolyte

\[ E_{\text{CELL}} = k' - 0.059 \log P_{\text{NH}_3} \]

Limitations:

- excellent sensitivity over ions \textbf{BUT} poor selectivity over other gases
- \( \text{CO}_2 \) and \( \text{SO}_2 \) cannot be measured simultaneously

Use buffered inner filling solutions (pH constant) and use inner ISE detector selective towards an ionic form of the gas.

(limitation at high gas levels due to changes in pH)


**ISE Enzyme sensors**

**Biological specificity of enzyme catalyzed reaction + selectivity of the ISE**

Enzymes accelerate chemical reactions by a factor $10^8$-$10^{20}$ compared with uncatalyzed reactions!

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Enzyme</th>
<th>Reaction</th>
<th>ISE detectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>Urease</td>
<td>$\text{Urea} \rightarrow 2\text{NH}_3 + \text{CO}_2$</td>
<td>$\text{NH}_4^+$-glass or polymer, $\text{NH}_3$-gas sensor, $\text{H}^+$-glass or polymer, $\text{NH}_3$-gas</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Creatininase</td>
<td>$\text{Creatinine} \rightarrow \text{N-methylhydrantoin} + \text{NH}_3$</td>
<td>$\text{NH}_4^+$-glass or polymer, $\text{NH}_3$-gas</td>
</tr>
<tr>
<td>L-glutamate</td>
<td>Glutamate decarboxylase</td>
<td>$\text{L-glutamate} \rightarrow \text{GABA} + \text{CO}_2$</td>
<td>$\text{CO}_2$-gas sensor</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>$\beta$-Glucosidase</td>
<td>$\text{Amygdalin} \rightarrow \text{HCN} + 2\text{C}_6\text{H}_1\text{O}_6$ + benzaldehyde</td>
<td>$\text{CN}^-$-solid-state</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glucose oxidase</td>
<td>$\text{Glucose} + \text{O}_2 \rightarrow \text{gluconic acid} + \text{H}_2\text{O}_2$</td>
<td>$\text{H}^+$-glass or polymer</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Penicillinase</td>
<td>$\text{Penicillin} \rightarrow \text{penicilloic acid}$</td>
<td>$\text{H}^+$-glass or polymer</td>
</tr>
</tbody>
</table>
ISE Enzyme sensors: Urea Sensor

Physically or chemically Immobilized enzyme at the surface of a ISE

Urea example (Urease + Ammonium ISE)

\[
\text{Urease} \\
\text{Urea} + H_2O \rightarrow 2NH_3 + CO_2
\]

At physiological pH (~7.4)

\[
\begin{align*}
NH_3 & \Rightarrow NH_4^+ \\
CO_2 & \Rightarrow HCO_3^-
\end{align*}
\]

Detection limit: membrane detection limit + mass transfer into the enzymatic layer

0.01mM – 10mM
**ISE DNA hybridization sensor**

Detection of hybridization between complementary ss oligonucleotides on the phase boundary PVC membrane/solution

Detection principle: the hybridization process of single-stranded oligonucleotides close to a PVC membrane induces a measurable redistribution of the ion concentration within intermolecular regions

Outline.

- Amperometric transducing principle
- Faradaic vs nonfaradaic process
- Charging currents in electrochemical measurements
- Electron transfer at electrode/solution interface
  - Mass transfer controlled reactions
  - Kinetic controlled reactions
- Amperometric techniques
- Applications
Amperometric Transducing Principle

Potentiometric versus Amperometric Transducing

- The current (electrons, $n_e$, crossing the electrode/electrolyte interface) is the measured parameter

$$i(A) = \frac{dQ}{dt} \left( \frac{\text{Coulombs}}{s} \right)$$

$Q \equiv q \cdot n_e \equiv \text{total charge}$

Faraday law → consumption or production of 1mol requires 96485.4 C (Faraday constant) in a one e$^-$ reaction

$$N(\text{mol electrolysed}) = \frac{Q(\text{Coulombs})}{nF(\text{Coulombs/mol})}$$

$$\text{Rate (mol/s)} = \frac{dN}{ds} = \frac{i}{nF}$$
Faradaic versus Nonfaradaic current process

**Faradaic current process**
Charges are transferred across the electrode/electrolyte interface, producing oxidation or reduction processes.

**Nonfaradaic current process**
No charge cross the interface, though external transient currents can flow.
Adsorption/desorption changing the structure of the electrode/electrolyte interface.

charging current
Charging currents in electrochemical measurements

a) Potential step

\[ i = \frac{dq}{dt} \]

\[ E = E_S + E_C = iR_S + \frac{q}{C_d} \]

\[ \frac{dq}{dt} = -\frac{q}{R_S C_d} + \frac{E}{R_S} \]

\[ q = EC_d \left[ 1 - e^{-t/R_S C_d} \right] \rightarrow i = \frac{E}{R_S} e^{-t/R_S C_d} \]
Charging currents in electrochemical measurements

b) Current step

\[ q = \int idt \quad E = E_S + E_C = iR_s + \frac{i}{C_d} \int_0^t dt \]

\[ E = i \left( R_s + \frac{t}{C_d} \right) \]

c) Voltage ramp

\[ E = \nu t = R_s \left( dq/dt \right) + \frac{q}{C_d} \]

\[ i = \nu C_d \left( 1 - \exp \left( -t/R_s C_d \right) \right) \]
d) Cyclic linear potential sweep
Electron Transfer at electrode/solution interface

Factors affecting electrode reaction rates

1. Mass transfer
   Migration
   Diffusion
   Convection

2. Electron transfer

3. Chemical reactions

4. Other surface reactions
   adsorption
   desorption

\[ O + n e^- \leftrightarrow R \]

- When steady-state current is obtained, the rates of all reaction step are the same
- The magnitude of the current is limited by the slowest process: rate-determining step
- The rate constant of some of the processes depends upon the potential