Electrochemical Biosensors - theory part 1

WEEK 1

Fall Semester

Faculty: Dr. Javed H. Niazi KM
Faculty of Engineering & Natural Sciences
Sabanci University
Topics that will be covered in the course

- History of biosensor development, applications and requirements of biosensors and classification

- Principles of molecular recognition and transduction signal acquisition
  - Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
  - Design considerations for use of recognition elements in biosensors
  - Modeling of reactions for various biosensor applications - electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.

- Modification of sensor surfaces and immobilization techniques
  - Covalent modification of surfaces using surface chemistry
  - Self Assembled Monolayers (SAM) and adsorptions
  - Other ways to immobilize biological macromolecules on various solid surfaces

- Detection methods and Physical Sensors
  - Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
  - Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.

- Fabrication of biosensors
  - Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
  - Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces

- Data acquisition, statistical and error analysis
  - Inter and Intra-assays and Coefficient of variation (CV)
  - Signal to noise ratio
  - Normalization/optimization and signal retrieval

- Examples of commercial biosensors
Sources of Biological recognition elements

- **Bacteria**
  - Proteins
    - Enzymes
  - Nucleic acids
    - DNA
  - Carbohydrates
    - Oligo-Saccharides
    - Polysacharides
    - Glycans
    - Cellulosics
  - Lipids
    - Lipid membrane
    - Liposomes
    - micelles
  - Whole cells
    - Bacteria

- **Fungi**
  - Proteins
  - Nucleic acids
    - DNA
    - RNA
    - PNA
  - Carbohydrates
    - Oligo-Saccharides
    - Polysacharides
    - Glycans
  - Lipids
    - Lipid membrane
    - Liposomes
    - micelles
  - Whole cells
    - Fungi

- **Human/animal**
  - Proteins
  - Nucleic acids
    - DNA
    - RNA
    - PNA
  - Carbohydrates
    - Oligo-Saccharides
    - Polysacharides
    - Glycans
    - Cellulosics
  - Lipids
    - Lipid membrane
    - Liposomes
    - micelles
  - Whole cells
    - Human

- **Virus**
  - Proteins
  - Nucleic acids
    - DNA
    - RNA
    - PNA
  - Carbohydrates
    - Oligo-Saccharides
    - Polysacharides
    - Glycans
  - Lipids
    - Lipid membrane
    - Liposomes
    - micelles
  - Whole cells
    - Virus
Biosensor Recognition Elements

Enzymes: eg., glucose oxidase for detection/measurement of glucose
Typical enzyme isolation from cells

Ammonium Sulfate Precipitation Table:

<table>
<thead>
<tr>
<th>Initial concentration of ammonium sulfate</th>
<th>Solid ammonium sulfate (grams) to be added to 1 liter of solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0.56</td>
</tr>
<tr>
<td>10</td>
<td>0.8</td>
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<td>15</td>
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<td>20</td>
<td>1.26</td>
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<td>1.56</td>
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<td>2.56</td>
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<td>3.56</td>
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<td>85</td>
<td>10</td>
</tr>
<tr>
<td>90</td>
<td>11</td>
</tr>
<tr>
<td>95</td>
<td>12</td>
</tr>
</tbody>
</table>

6000-7000 different proteins in a cell
Avogadro's number and the mole

Avogadro's number = \(6.02 \times 10^{23}\) atoms/molecules/particles

<table>
<thead>
<tr>
<th>Name of Substance</th>
<th>Formula</th>
<th>Formula Weight (amu)</th>
<th>Molar Mass (g/mol)</th>
<th>Number and Kind of Particles in One Mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic nitrogen</td>
<td>N</td>
<td>14.0</td>
<td>14.0</td>
<td>(6.02 \times 10^{23}) N atoms</td>
</tr>
<tr>
<td>Molecular nitrogen</td>
<td>(N_2)</td>
<td>28.0</td>
<td>28.0</td>
<td>(6.02 \times 10^{23}) (N_2) molecules</td>
</tr>
<tr>
<td>Silver</td>
<td>Ag</td>
<td>107.9</td>
<td>107.9</td>
<td>(6.02 \times 10^{23}) Ag atoms</td>
</tr>
<tr>
<td>Silver ions</td>
<td>(Ag^+)</td>
<td>107.9(^a)</td>
<td>107.9</td>
<td>(6.02 \times 10^{23}) (Ag^+) ions</td>
</tr>
<tr>
<td>Barium chloride</td>
<td>BaCl(_2)</td>
<td>208.2</td>
<td>208.2</td>
<td>(6.02 \times 10^{23}) BaCl(_2) units</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.02 \times 10^{23}) (Ba^{2+}) ions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2(6.02 \times 10^{23})) (Cl^-) ions</td>
</tr>
</tbody>
</table>

\(^a\)Recall that the electron has negligible mass; thus, ions and atoms have essentially the same mass.

- One mole of atoms, ions, or molecules contains Avogadro's number of those particles.
- One mole of molecules or formula units contains Avogadro's number times the number of atoms or ions of each element in the compound
Electrochemistry -

a. branch of chemistry that studies chemical reactions and processes in which electric charges are involved
b. Transfer of an electron from a species in solution to an electrode, or vice versa
c. Common in the analytical field and has resulted in the development of:
   - Potentiometry
   - Voltammetry (amperometry)
   - Coulometry - an electrolysis reaction by measuring the amount of electricity (in coulombs) consumed or produced

Where there is oxidation, there is reduction

Substance oxidized loses electron(s)
Substance reduced gains electron(s)

Conventional current flow is opposite to electron flow

eecs.oregonstate.edu/~traylor/ece112/.../elect_flow_vs_conv_I.pdf
Electrochemical Biosensor

There are three basic electrochemical processes - useful in transducers for biosensors:

i. **Potentiometry** - the measurement of a cell potential at zero current

ii. **Voltammetry (amperometry)** - an oxidizing (or reducing) potential is applied between the cell electrodes and the cell current is measured

iii. **Conductimetry** - the conductance (reciprocal of resistance) of the cell is measured by an alternating current bridge method.

<table>
<thead>
<tr>
<th>POTENTIOMETRY</th>
<th>AMPEROMETRY</th>
<th>VOLTAMMETRY</th>
</tr>
</thead>
<tbody>
<tr>
<td>✷ Measure potential difference (E) at zero current</td>
<td>✷ Measure current at fixed potential</td>
<td>✷ Measure current as a function of scanned potential</td>
</tr>
<tr>
<td>✷ Two electrodes are used – indicator and reference</td>
<td>✷ Two or three electrodes are used</td>
<td>✷ Three electrodes are used – working, reference and counter (auxiliary)</td>
</tr>
<tr>
<td>✷ Carried out under equilibrium conditions</td>
<td>✷ Coulometry – when the current is integrated to give total charge</td>
<td>✷ Non-equilibrium measurement – gives kinetic information</td>
</tr>
<tr>
<td>✷ Quantitative analysis – E is related to the concentration of ions in the sample</td>
<td></td>
<td>✷ Qualitative and quantitative analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>✷ Polarography – use of a dropping Hg electrode</td>
</tr>
</tbody>
</table>
i. Potentiometry
1. Potentiometry - principle

A metal electrode dipped in electrolyte solution (one half cell)

- If a piece of metal is placed in an electrolyte solution, there is charge separation b/w metal (electrode) and the solution.
- Sets up an electron pressure, usually called a potential.
- It cannot be measured directly - requires a combination of two such electrode-electrolyte solution combinations.
- Each is called a half-cell.

Two half-cell electrodes combined, making a complete cell

- Two half cells -connected by means of an electrically conducting bridge or membrane
- Two electrodes are connected externally by a potential measuring device (digital voltmeter, DVM).
- DVM has a very high internal impedance (≈10^{12} \Omega) - such that very little current will flow through it.
- If the voltage to be measured is 1V, then the Ohm's law (V=IR), current \( I = 10^{-12} \text{ A} \) (1pA)
- The electrical circuit is now complete and the e.m.f. of the cell can be measured.
- This value is the difference between the electrode potentials of the two half-cells.
Electromotive force (e.m.f)

- Water only spontaneously flows one way in a waterfall.
- Likewise, electrons only spontaneously flow one way in a redox reaction: from higher to lower potential energy.

The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

It is also called the cell potential, and is designated $E_{\text{cell}}$. 
To make a current flow through a resistance there must be a voltage across that resistance. Ohm's Law shows the relationship between the voltage (V), current (I) and resistance (R). It can be written in three ways:

\[ V = I \times R \quad \text{(or)} \quad I = \frac{V}{R} \quad \text{(or)} \quad R = \frac{V}{I} \]

where:
- \( V \) = voltage in volts (V)
- \( I \) = current in amps (A)
- \( R \) = resistance in ohms (\( \Omega \))

Voltage can be thought of as the pressure pushing charges along a conductor, while the electrical resistance of a conductor is a measure of how difficult it is to push the charges along.

Voltage is the Cause, Current is the Effect

Voltage attempts to make a current flow, and current will flow if the circuit is complete. Voltage is sometimes described as the 'push' or 'force' of the electricity, it isn't really a force but this may help you to imagine what is happening. It is possible to have voltage without current, but current cannot flow without voltage.

Voltage, V

- Voltage is a measure of the energy carried by the charge. 
  Strictly: voltage is the “energy per unit charge”.
- The proper name for voltage is potential difference or p.d. for short, but this term is rarely used in electronics.
- Voltage is supplied by the battery (or power supply).
- Voltage is used up in components, but not in wires.
- We say voltage across a component.
- Voltage is measured in volts, V.
- Voltage is measured with a voltmeter, connected in parallel.
- The symbol V is used for voltage in equations.
Daniell cell - an example for potentiometry

If we consider each half-cell -> reaction for each half-cell is:

\[
\begin{align*}
\text{Cu}^{2+} + 2e^- &= \text{Cu} \quad \text{(half-cell electrode reaction 1)} \\
\text{Zn}^{2+} + 2e^- &= \text{Zn} \quad \text{(another reaction 2)}
\end{align*}
\]

If we subtract reaction equation 2 from 1 we obtain

\[
\text{Cu}^{2+} + \text{Zn} = \text{Cu} + \text{Zn}^{2+}
\]

The Gibbs free energy (\(\Delta G\)) for this reaction is negative (spontaneous in the direction shown).

The \(\Delta G\) is simply related to the e.m.f of the cell:

\[
\Delta G = -nFE
\]

- \(n\)-No. of electrons transferred (here \(n = 2\))
- \(F\) - is Faraday constant = 96,487 C/mol
- \(E\) - is the e.m.f of the cell

What the \(\Delta G\) values are for reactions 1 and 2 separately?

Observed electrode volt
\(E_{obs} = 1.10\ V\)
Hydrogen electrode (separate measurement of std. electrode potential of one half-cell)

- If $\Delta G_1$ and $\Delta G_2$, we could find $E_1$ and $E_2$ separately.
- Hydrogen electrode provides separation of $E_1$ and $E_2$.

Hydrogen is not a metal but it can be oxidized to $H^+$ by the removal of an electron:

\[
H - e^- = H^+
\]

Also written as: \( H^+ + e^- = \frac{1}{2}H_2 \)

$\Delta G$ for this reaction is ZERO

The std. state being with $[H^+] = 1\text{M}$, partial pressure of $H_2 = 1$ and temp = 298 K (25 °C).

The Gibbs free energy is designated $\Delta G^0$

The std. electrode potential for hydrogen is therefore:

\[
E_{H^+/H_2}^0 = 0
\]
Half-cell Potential (single electrode)

Practical- half-cell of **hydrogen** electrode: this can be combined with any other half-cell electrode

\[ \text{Eg.,} \quad \text{Cu}^{2+} + 2e^- = \text{Cu} \quad E_1 \]
\[ 2\text{H}^+ + 2e^- = \text{H}_2 \quad E_H (=0) \]

Subtracting eqn. 4 from eqn. 3:

\[ \text{Cu}^{2+} + \text{H}_2 = \text{Cu} + 2\text{H}^+ \]

Thus \( E_{\text{cell}} = E_1 - E_H = +0.34 \text{ V} \)

Therefore, \( E^0_{\text{Cu}} = +0.34 \text{ V} \) (one half)

The other half of the Daniel Cell, the **zinc** electrode:

\[ \text{Zn}^{2+} + 2e^- = \text{Zn} \quad E_{\text{Zn}} \]
\[ 2\text{H}^+ + 2e^- = \text{H}_2 \quad E_H (=0) \]

Subtracting eqn. 6 from eqn. 5:

\[ \text{Zn}^{2+} + \text{H}_2 = \text{Zn} + 2\text{H}^+ \]

Thus \( E_{\text{cell}} = E_{\text{Zn}} - E_H = -0.76 \text{ V} \)

Therefore, \( E^0_{\text{Zn}} = -0.76 \text{ V} \) (other half)

Combining two half-cell e.m.f.s for copper and zinc gives the cell e.m.f. for the Daniel cell

\[ E_{\text{cell}} = +0.34 - (-0.76) = 1.10 \text{ V} \]
Cell Potential

Cell potential is measured in volts (V).

\[ 1 \text{ V} = 1 \frac{\text{J}}{\text{C}} \]

Standard Cell Potentials - The cell potential at standard conditions can be found through this equation:

\[ E_{\text{cell}}^\circ = E_{\text{red (cathode)}}^\circ - E_{\text{red (anode)}}^\circ \]

Because cell potential is based on the potential energy per unit of charge, it is an intensive property.

\[ \text{e.m.f (V)} = \text{Work/energy (Joule, J)}/\text{Charge (Columb, C)} \]

\[ E_{\text{cell}}^\circ = E_{\text{red (cathode)}}^\circ - E_{\text{red (anode)}}^\circ \]

\[ = +0.34 \text{ V} - (-0.76 \text{ V}) \]

\[ = +1.10 \text{ V} \]

\[ \begin{array}{c}
\text{Cu}^{2+} + 2e^- \rightarrow \text{Cu} (s) \quad E^\circ = +0.340 \\
\text{Zn}^{2+} + 2e^- \rightarrow \text{Zn} (s) \quad E^\circ = -0.763 \\
\text{Cu}^{2+} + \text{Zn} (s) \rightarrow \text{Cu} (s) + \text{Zn}^{2+} \quad E_{\text{cell}}^\circ = +1.108 \text{ V}
\end{array} \]
Electron transfer through electrode/solution interface - A schematic representation

Electrolyte

\[ \text{Zn} \rightarrow \text{Zn}^{2+} + 2e^- \quad \text{Cu}^{2+} + 2e^- \rightarrow \text{Cu} \]
Standard Electrode Potential (E°) for few reactions

<table>
<thead>
<tr>
<th>Half-reaction</th>
<th>E°, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li⁺(aq) + e⁻ → Li(s)</td>
<td>-3.040</td>
</tr>
<tr>
<td>K⁺(aq) + e⁻ → K(s)</td>
<td>-2.924</td>
</tr>
<tr>
<td>Na⁺(aq) + e⁻ → Na(s)</td>
<td>-2.713</td>
</tr>
<tr>
<td>Al³⁺(aq) + 3e⁻ → Al(s)</td>
<td>-1.676</td>
</tr>
<tr>
<td>Zn²⁺(aq) + 2e⁻ → Zn(s)</td>
<td>-0.763</td>
</tr>
<tr>
<td>Fe²⁺(aq) + 2e⁻ → Fe(s)</td>
<td>-0.440</td>
</tr>
<tr>
<td><strong>2H⁺(aq) + 2e⁻ → H₂(g)</strong></td>
<td><strong>0.000</strong></td>
</tr>
<tr>
<td>Cu²⁺(aq) + e⁻ → Cu(s)</td>
<td>+0.340</td>
</tr>
<tr>
<td>I₂(s) + 2e⁻ → 2I⁻</td>
<td>+0.535</td>
</tr>
<tr>
<td>Ag⁺(aq) + e⁻ → Ag(s)</td>
<td>+0.800</td>
</tr>
<tr>
<td>Br₂(l) + 2e⁻ → 2Br⁻(aq)</td>
<td>+1.065</td>
</tr>
<tr>
<td>Cl₂(g) + 2e⁻ → 2Cl⁻(aq)</td>
<td>+1.358</td>
</tr>
<tr>
<td>Au⁺ + e⁻ → Au(s)</td>
<td>+1.680</td>
</tr>
<tr>
<td>F₂(g) + 2e⁻ → 2F⁻(aq)</td>
<td>+2.866</td>
</tr>
</tbody>
</table>
Three electrode system

Components of Electrochemical cell

Three electrodes:
Working, Counter, and Reference

An electrolyte solution:
Solvent, supporting

Membrane: O or X

Potentiostat: e⁻ delivery of, or redox reactions
Involves the transmission of ions

The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

It is also called the cell potential, and is designated $E_{cell}$. 

Cathode is the working/indicator electrode. (right half-cell). - Anode is the counter/reference electrode. (left half-cell).
Potentiometer

• A device for measuring the potential of an electrochemical cell without drawing a current or altering the cell’s composition.

Potentiometric measurements:

Potentiometric measurements are made using a potentiometer to determine the difference in potential between a **working** (an indicator) electrode and a **counter** (a reference) electrode.

- Cathode is the working/indicator electrode. (right half-cell)
- Anode is the counter/reference electrode. (left half-cell)
Potentiometric vs. Potentiostatic Methods

**Amperometry:** Changing current as a function of time - 
*A variable current condition*

*Current vs. [analyte]*

**Potentiometry:** Changing voltage as a function of time - 
*A zero (0) current condition*

*Voltage vs. [analyte]*
Principles of Potentiometry

✧ The cell voltage, $E_{\text{cell}}$, of a galvanic cell is measured at $I = 0$, i.e. when the electrochemical system is at equilibrium.

✧ $E_{\text{cell}}$ is governed by the electrode potential of the indicator electrode, $E_{\text{IE}}$

✧ $E_{\text{IE}}$ responds to the activity, $a_i$, of the analyte

✧ $a_i$ is related to the concentration, $c_i$, of an analyte

✧ $E_{\text{cell}}$ and $E_{\text{IE}}$ depend on $a_{\text{reactants}}$ and $a_{\text{products}}$ of the electrode reactions

✧ The $E_{\text{cell}}$ or $E_{\text{IE}}$ are related to the activities of the reaction species by the NERNST equation:

$$E = E^\circ + \left( \frac{RT}{nF} \right) \ln \left( \frac{a_{\text{ox}}}{a_{\text{red}}} \right)$$
\[ E = E^\circ + \left( \frac{RT}{nF} \right) \ln \left( \frac{a_{\text{ox}}}{a_{\text{red}}} \right) \]

- \( E^\circ \) is the **formal potential** – the potential of the half cell (vs. a reference system) when \( a_{\text{ox}}/a_{\text{red}} = 1 \) and all other species are present at specified concentrations (e.g. specified pH and electrolyte).
- \( R \) is the molar gas constant, 8.314 J K\(^{-1}\) mol\(^{-1}\)
- \( T \) is the absolute temperature (K)
- \( n \) is the number of electrons transferred
- \( F \) is Faraday’s constant, 96500 C mol\(^{-1}\)
- \( a_{\text{ox}}/a_{\text{red}} \) is the ratio of the activity of the oxidized and reduced forms of the analyte

For infinitely dilute solutions (\( C_i < 10^{-1} \) M) \( a_i \approx C_i \).
In potentiometric sensors, the zero-current potential (relative to a reference) developed at a selective membrane or electrode surface in contact with a sample solution is related to analyte concentration (logarithmic relationship between measured potential and analyte concentration).

The main use of potentiometric transducers in biosensors: a pH-stat (the reactions consume or produce protons).

The biosensor consists of an immobilized enzyme membrane surrounding the probe from a pH-meter:

- **Mechanism:** pH changes cause changes in enzyme activity; pH maintained at a constant value by the addition of acid or base → the rate of titrant addition proportional to the rate of enzymatic reaction (specially within low buffer concentration!)

- **Three types of ion-selective electrodes** which are of use in biosensors:
  - Glass electrodes for cations (typical potentiometric biosensors)
  - Glass pH electrodes coated with a gas-permeable membrane selective for CO$_2$, NH$_3$ or H$_2$S.
  - The iodide electrode is useful for the determination of I$^-\text{ or CN}^-$ in the peroxidase reaction in penicillinase reaction mediated with I$^-\text{ or CN}^-$. 
A substance that modifies the transition state to lower the activation energy is termed a \textit{catalyst}; a \textit{biological catalyst} is termed an \textit{enzyme}.

\[ \text{Catalytic antibodies involved} \]

\textbf{The reaction:} hydrolytic cleavage of the ester linkage of phenyl acetate.

Ab trapped at the surface of a pH electrode using a dialysis membrane will bind to the transition state of the hydrolysis reaction.

\textbf{Figure 7.3.} Transition state analogue hapten used for the generation of catalytic antibodies for phenyl acetate hydrolysis.
- Ex) for phenyl acetate as a model for the evaluation of catalytic antibodies:

- **Mechanism of detection:** Ab trapped at the surface of a pH electrode using a dialysis membrane will bind to the transition state of the hydrolysis reaction $\rightarrow$ the reaction produce a change in local pH at the surface of the electrode **due to an acetic acid as a product** $\rightarrow$ The measured pH decreases as the phenyl acetate concentration increases.

- Selectivity: a number of similar compounds produce signal, particularly those containing RC OOC6H5 group but the selectivity similar to the corresponding hydrolytic enzyme.

- A novel use of Abs as chemical recognition agents!

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**Figure 7.4.** (a) Diagram of phenyl acetate sensor and (b) calibration curve.
POTENTIOMETRIC BIOSENSORS [II]

- The best known potentiometric sensor is the Ion Selective Electrode (ISE).

- ISEs used in conjunction with immobilised enzymes can serve as the basis of electrodes that are selective for specific enzyme substrates.

- The two main ones are for urea and creatinine - These potentiometric enzyme electrodes are produced by entrapment the enzymes urease and creatinase, on the surface of a cation sensitive ($\text{NH}_4^+$) ISE.

- Solvent polymeric membrane electrodes are commercially available and routinely used for the selective detection of several ions such as $\text{K}^+$, $\text{Na}^+$, $\text{Ca}^{2+}$, $\text{NH}_4^+$, $\text{H}^+$, $\text{CO}_3^{2-}$) in complex biological matrices.

- The antibiotics nonactin and valinomycin serve as neutral carriers for the determination of $\text{NH}_4^+$ and $\text{K}^+$, respectively.
POTENTIOMETRIC BIOSENSORS

- \( E = E_0 + RT/nF \ln[\text{analyte}] \)
  - \( E_0 \) is a constant for the system, \( R \) is the universal gas constant, \( T \) is the absolute temperature, \( z \) is the charge number, \( F \) is the Faraday number
  - \( \ln[\text{analyte}] \) is the natural logarithm of the analyte activity.
  - \( E \) is measured potential (two electrodes system)

\[
\text{Urea} + \text{H}_2\text{O} + \text{H}^+ \xrightarrow{\text{urease}} 2\text{NH}_4^+ + \text{HCO}_3^-
\]

\[
\text{Creatinine} + \text{H}_2\text{O} \xrightarrow{\text{creatininase}} \text{N-methylhydantoin} + \text{NH}_4^+
\]

\[
\text{Penicillin} \xrightarrow{\text{penicillinase}} \text{Penicillonic Acid}
\]

In contact with pH electrode.

- Ag/AgCl reference electrode
- Internal aqueous filling solution
- Liquid ion exchanger
- Membrane/salt bridge
- Porous membrane containing ionophore
POTENTIOMETRIC BIOSENSORS - immunosensor

Ex) for digoxin:

- Employs a potassium (K+) ion-selective electrode (ISE) as the transducer.
- Chemical recognition: anti-digoxin Ab and a digoxin-crown ether conjugate.
- PVC [a poly(vinyl chloride)] membrane – separate internal K+ solution from an external layer of digoxin Ab trapped at the sensor surface.
- **Mechanism:** competition between free digoxin (analyte) crossing the dialysis membrane freely and the digoxin-crown conjugate at the PVC-aqueous interface → change in the transport of K+ across the PVC membrane (Ab-digoxin-crown held at the interface!)

![Diagram of digoxin immunosensor](image)

**Figure 7.5.** (a) Digoxin immunosensor and (b) calibration curves for digoxin.
Potentiometric Immunosensor

- employs K⁺ ion-selective electrode as a transducer
- chemical recognition involves anti-digoxin Ab & digoxin-crown ether conjugate
- PVC membrane separates an internal K⁺ ion from external layer of digoxin-Ab-trapped at sensor surface with a dialysis membrane.
- The digoxin-crown ether conjugate - selectively binds K⁺ present only in the PVC layer - hydrophobic properties
- Competition by free digoxin (analyte) that freely crosses the dialysis membrane by the digoxin-crown conjugate at the PVC/aqueous interface for antibody-in the reaction causes a change in the transport of K⁺ across the membrane
- because the antibody-digoxin-crown conjugate is held at its
- interface

An ex. of competitive immunosensor - binding interaction to be monitored.