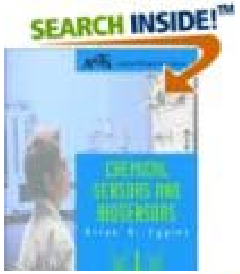
Biosensors

Synopsys

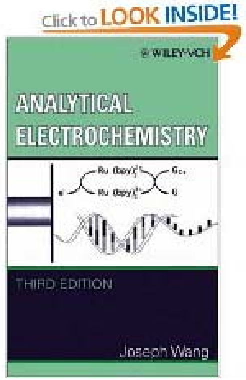
- Introduction to biosensors. Concepts and definitions. Electrochemical sensors: Potentiometry
- Electrochemical sensors: Potential-controlled techniques
- Electrochemical sensors: examples and applications
- DNA biosensors. Micro and nanoarrays
- SPR sensors: principle and instrumentation
- Reaction kinetics on the surface. Immobilization strategies.
- Nanotube and Nanowire based biosensors
- Optical biosensors

Biosensors

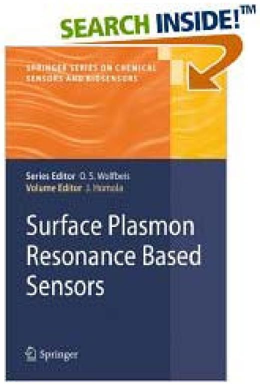
Course web site: **http://homes.nano.aau.dk/lg/Biosensors2009.htm**



- Brian R. Eggins “Chemical Sensors and Biosensors”, Wiley 2002

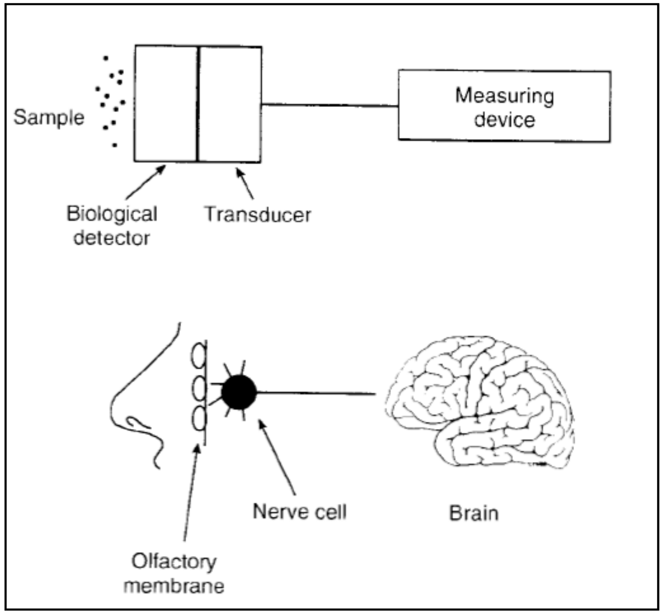


- J. Wang “Analytical electrochemistry”, 3rd ed. Wiley-VCH 2006



- Surface Plasmon Resonance Based Sensors, ed. By J. Homola, Springer 2006

Concept and Definitions



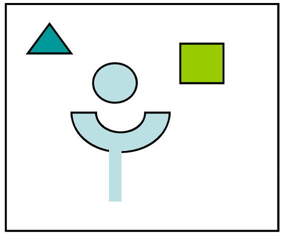
- ***Sensors is a device that detects or measures a physical property and records, indicates or otherwise responds to it*** (Oxford English Dictionary)
- Transducer is a device that converts an observed change into a **measurable** signal
- Actuator is device that produce a display *(or any other action)*

Sensors

- **Physical sensors**– measuring physical quantaties for their own sake ☺
- **Chemical sensors**– devices that responds to a particular analyte in a selective way through a chemical reaction and can be used for qualitative/quantitative determination of the analyte (R.W. Catterall)
- **Biosensors** – devices incorporating a biological sensing elements

Aspects of Sensors

Recognition Elements – enable sensor to respond selectively to a particular analyte or a group of those (e.g. ion-selective electrodes, enzymes, antibodies, nucleic acids etc.)



Immobilisation techniques: adsorption, entrapment, covalent attachment etc

Transducers

Electrochemical

Potentiometric – measuring the potential of a cell at zero current

Voltammetric – increasing/decreasing potential applied until oxidation/reduction of the substanced in question occurs. In **amperometric** mode the potential is set directly and the current is measured.

Conductometric – measuring change in conductance of the solution

FET based sensors – mainly potentiometric, but can be voltammetric or conductometric.

Optical (e.g absorbtion spectroscopy, fluorescent spectroscopy, SPR etc.)

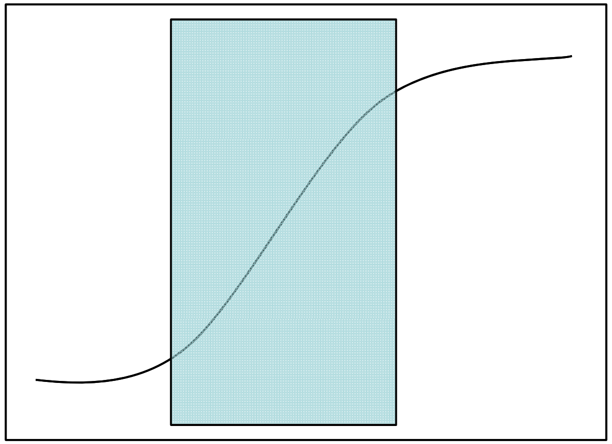
Piezo-electric

Thermal

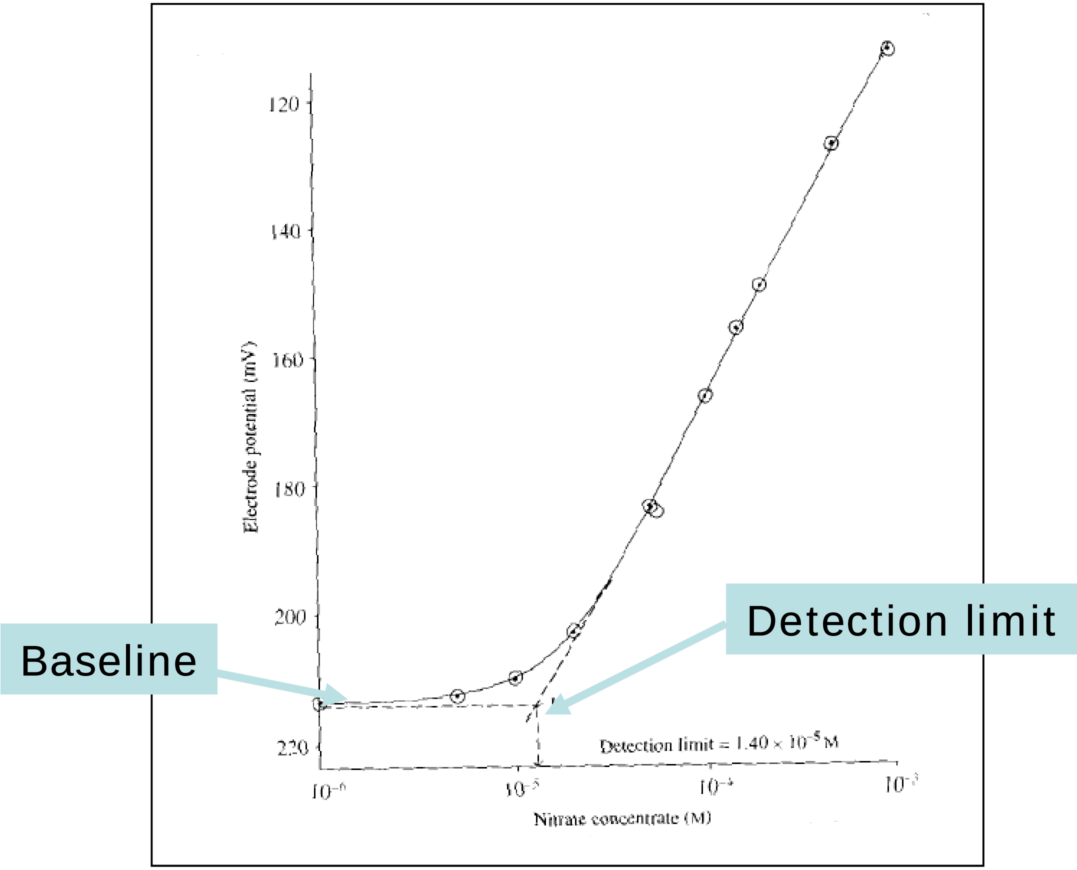
Performance factors

- **Selectivity** – ability to discriminate between different substances
- **Sensitivity** range (and dynamic range)
- **Accuracy** (usually <5%)
- **Environmental** condition (condition as pH, temperature, ionic strength etc.)
- **Response time**
- **Recovery time**
- **Working lifetime (and shelf time as well)**

Range, Linear Range and Detection Limits



detection range



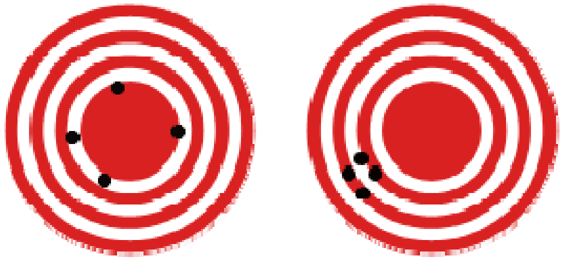
Baseline

Detection limit

- Detection limit: lowest detectable value, usually defined as the lowest limit of linear range.

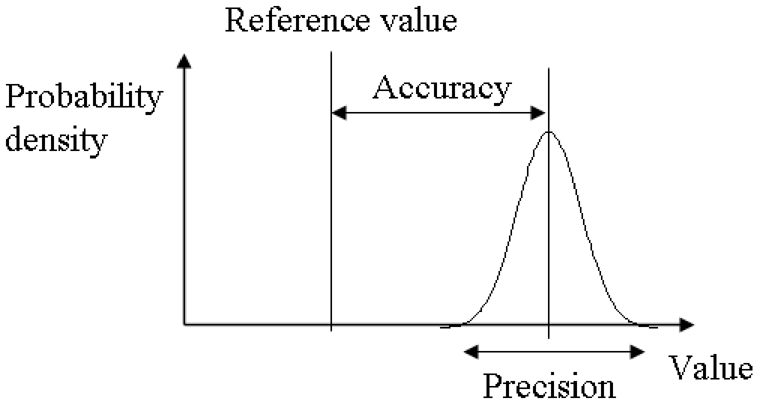
Precision, Accuracy, Repeatability and Reproducibility

- Precision – extent of random error in the measurement
- Accuracy – deviation of measurement from the actual value



Accurate, but not precise

Precise, but not accurate



- Repeatability – variation arising during repeating the exact measurement at the exactly the same condition within short time period
- Reproducibility – the same after longer period of time or at a different conditions.

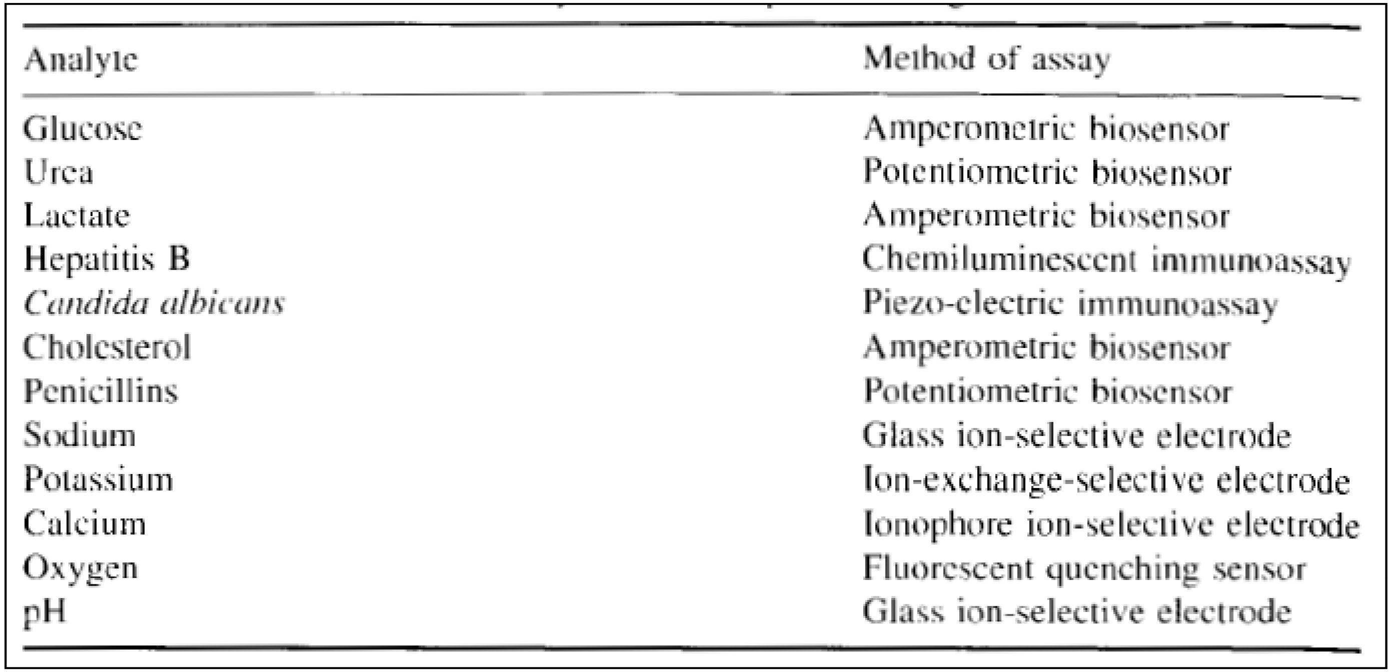
Time factors

- Response time – time necessary to come to an equilibrium and produce a reading
- Recovery time – time before the sensor is ready for another measurement
- Lifetime:
  - Degradation during continuous use
  - Storage in wet condition
  - Shelf life (in dry, in original packaging)

Areas of application

- **Health care** – measurements of blood, other biological fluids, ions, metabolites to show a patient metabolic state
- **Industrial processes**, e.g. Fermentation
- **Environmental monitoring**
- **Food control**
- **Warfare detection**

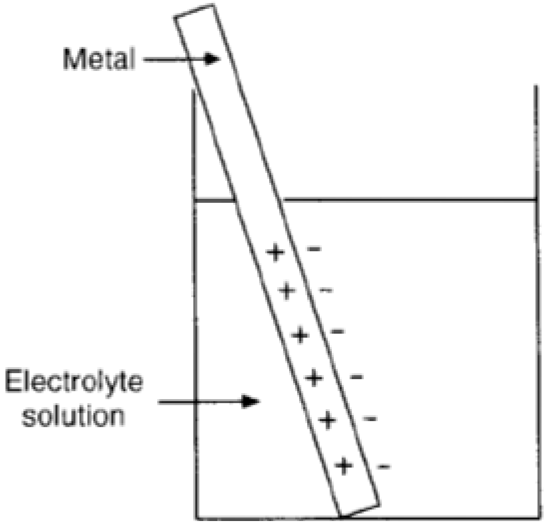
Common assays in medicine



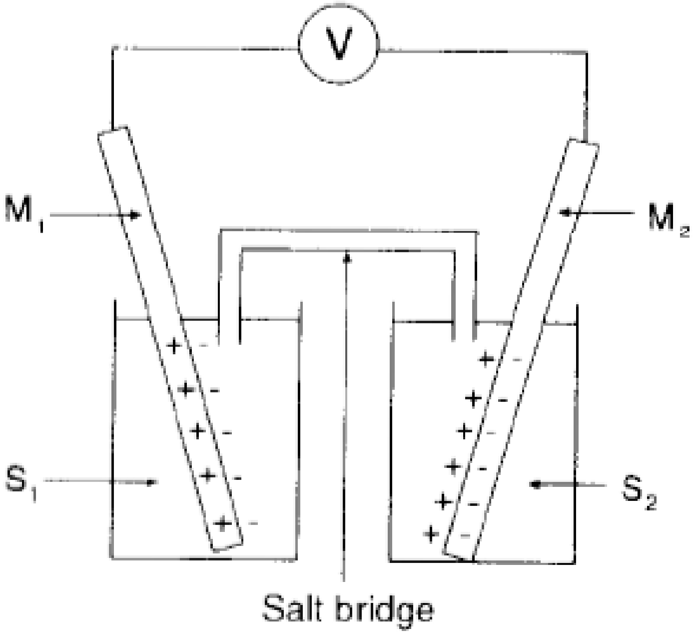
Analyte	Method of assay
Glucose	Amperometric biosensor
Urea	Potentiometric biosensor
Lactate	Amperometric biosensor
Hepatitis B	Chemiluminescent immunoassay
Candida albicans	Piezo-electric immunoassay
Cholesterol	Amperometric biosensor
Penicillins	Potentiometric biosensor
Sodium	Glass ion-selective electrode
Potassium	Ion-exchange-selective electrode
Calcium	Ionophore ion-selective electrode
Oxygen	Fluorescent quenching sensor
pH	Glass ion-selective electrode

Electrochemical Transducers

Cells and Electrodes



Half-cell



Electrochemical cell

<u>Voltage depends on:</u>

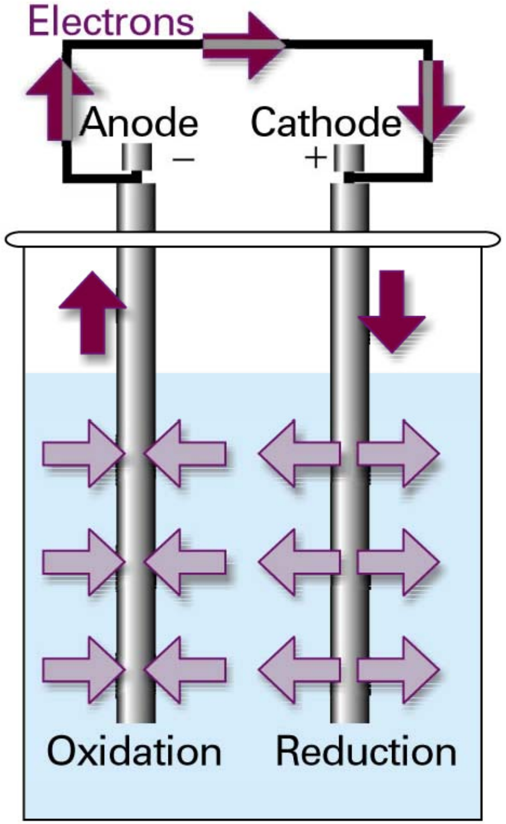
- Nature of electrodes
- Nature and concentration of solution
- Liquid junction potential (or potential across the membrane)

Equilibrium electrochemistry

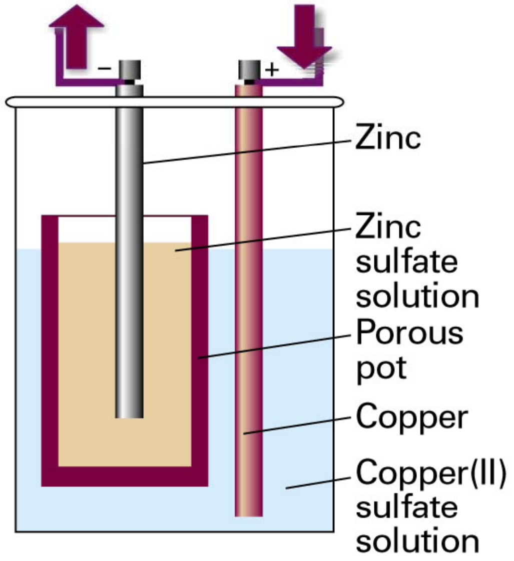
- Any redox reaction can be expressed as difference of two half-reactions, which are conceptual reactions showing gain of electrons

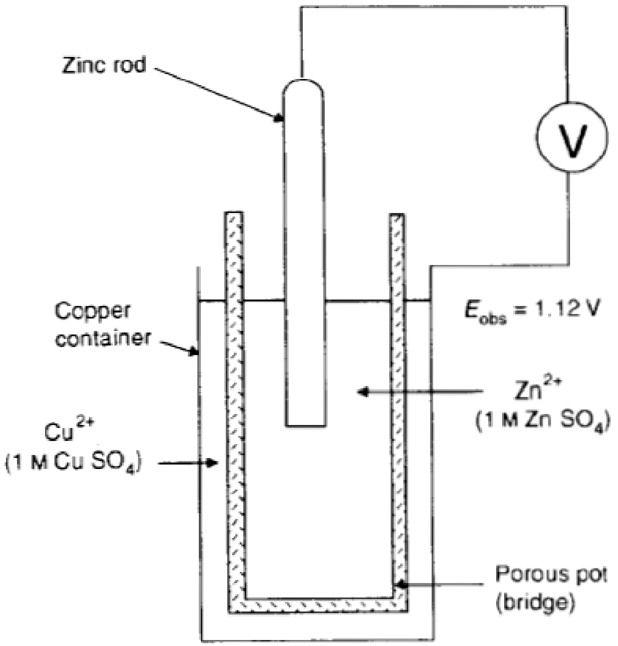
$$\mathrm{Ox} + \nu e^{-} \rightarrow \mathrm{Red}$$

The electrode where oxidation occurs is called **anode**, the electrode where reduction occurs is called **catode**.



Electrochemical cells





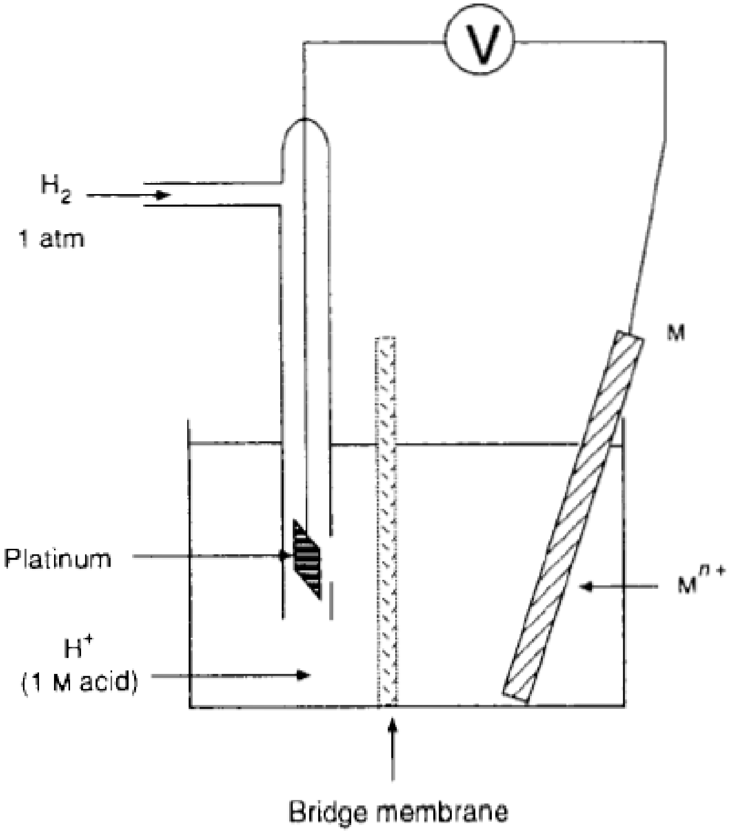
Notation:

$$Zn(s)\,|\,ZnSO_4(aq)\vdots CuSO_4(aq)\,|\,Cu(s)$$

Liquid junction

Can we find E for half-cell reaction? Not directly, but relative to RE.

Primary Reference Electrode



$$H^+ + e^- = 1/2\ H_2$$

- Standard Hydrogen Electrode (SHE) $[H^+]=1M$, $P(H_2)=1$ atm, T= 298K
- $\Delta G=0$
- Very reproducable (±10 μV)

$$Pt(s)\,|\,H_2(g)\,|\,H^+(aq) \qquad E^\theta = 0$$

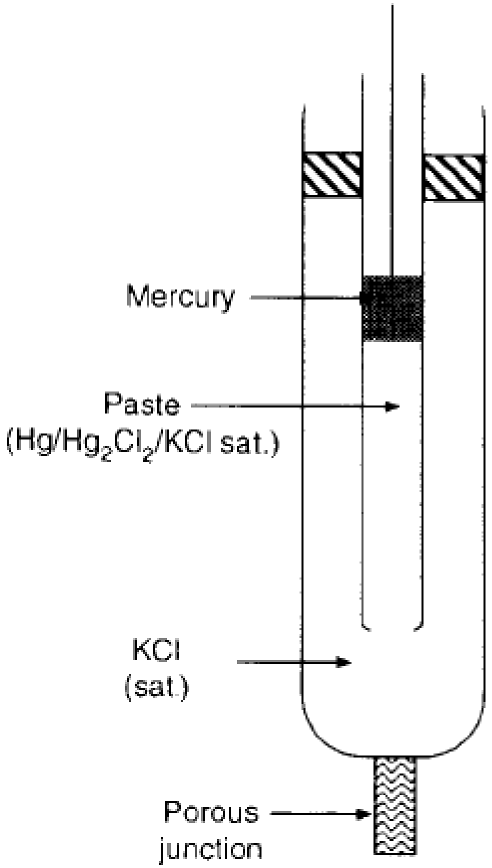
Secondary Reference electrodes

- Silver-Silver Chloride Electrode

$$AgCl + e^- = Ag + Cl^- \quad (E^0 = +0.22\ V)$$

- Saturated Calomel Electrode

$$Hg_2Cl_2 + 2e^- = 2Hg + 2Cl^- \quad (E^0 = 0.24\ V)$$



The Nernst equation

- A cell where overall cell reaction hasn't reached chemical equilibrium can do electrical work as the reaction drives electrons through an external circuit

$$w_{e,\max} = \Delta_r G$$

$$-\nu FE = \Delta_r G$$

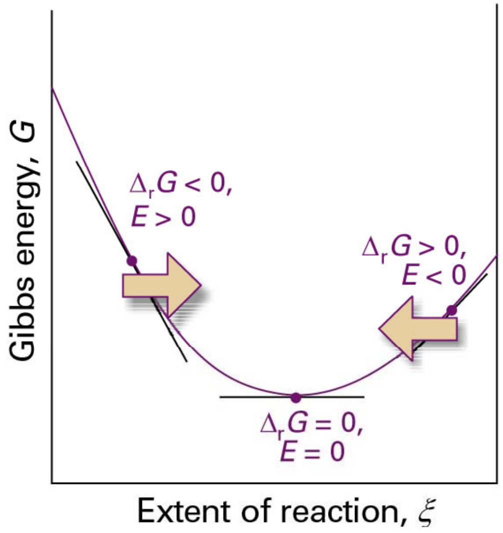
Number of electrons transferred

Faraday constant $F = eN_A$

Cell **emf**:

$$E = -\frac{\Delta_r G}{\nu F}$$

$$E = -\frac{\Delta_r G^{\ominus}}{\nu F} - \frac{RT}{\nu F}\ln Q = E^{\ominus} - \frac{RT}{\nu F}\ln Q$$



$$Q = \frac{\text{activities of products}}{\text{activities of reactants}} \qquad Q = \prod_j a_j^{\nu_j}$$

The Nernst equation

$$\mathrm{Ox} + ne^- = \mathrm{R}$$

8.314 J/K

$$E = E^0 + \frac{RT}{nF}\ln\left(\frac{a_{\mathrm{Ox}}}{a_{\mathrm{R}}}\right)$$

~0.059 log([Ox]/[R]) at 298K

- Special cases:

Metal in contact with its cations, e.g. $Cu|Cu^{2+}$:

$$E = E^{\ominus} + \frac{RT}{F}\ln a_{M^{n+}}$$

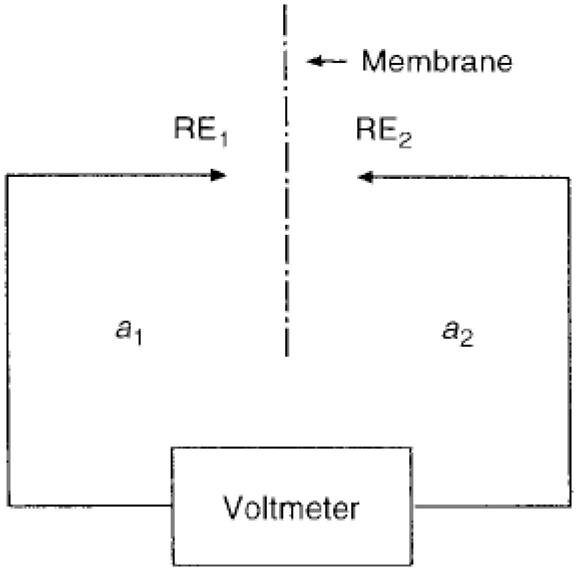
Gas in contact with its ions, e.g. $H_2|H^+$:

$$E = E^{\ominus} + \frac{RT}{F}\ln\frac{p_{H_2}^{1/2}}{a_{H^+}}$$

Metal electrode in contact with sparingly soluble salt, e.g. Hg|Hg2Cl2|Cl^-:

$$E = E^{\ominus} - \frac{RT}{F}\ln a_{Cl^-}$$

Concentration cell. Ion selective Electrodes



$$E_1 = E^0 + S\log[\mathrm{Ox}]_1$$

$$E_2 = E^0 + S\log[\mathrm{Ox}]_2$$

$$\Delta E = E_1 - E_2 = S\log([\mathrm{Ox}]_1/[\mathrm{Ox}]_2)$$

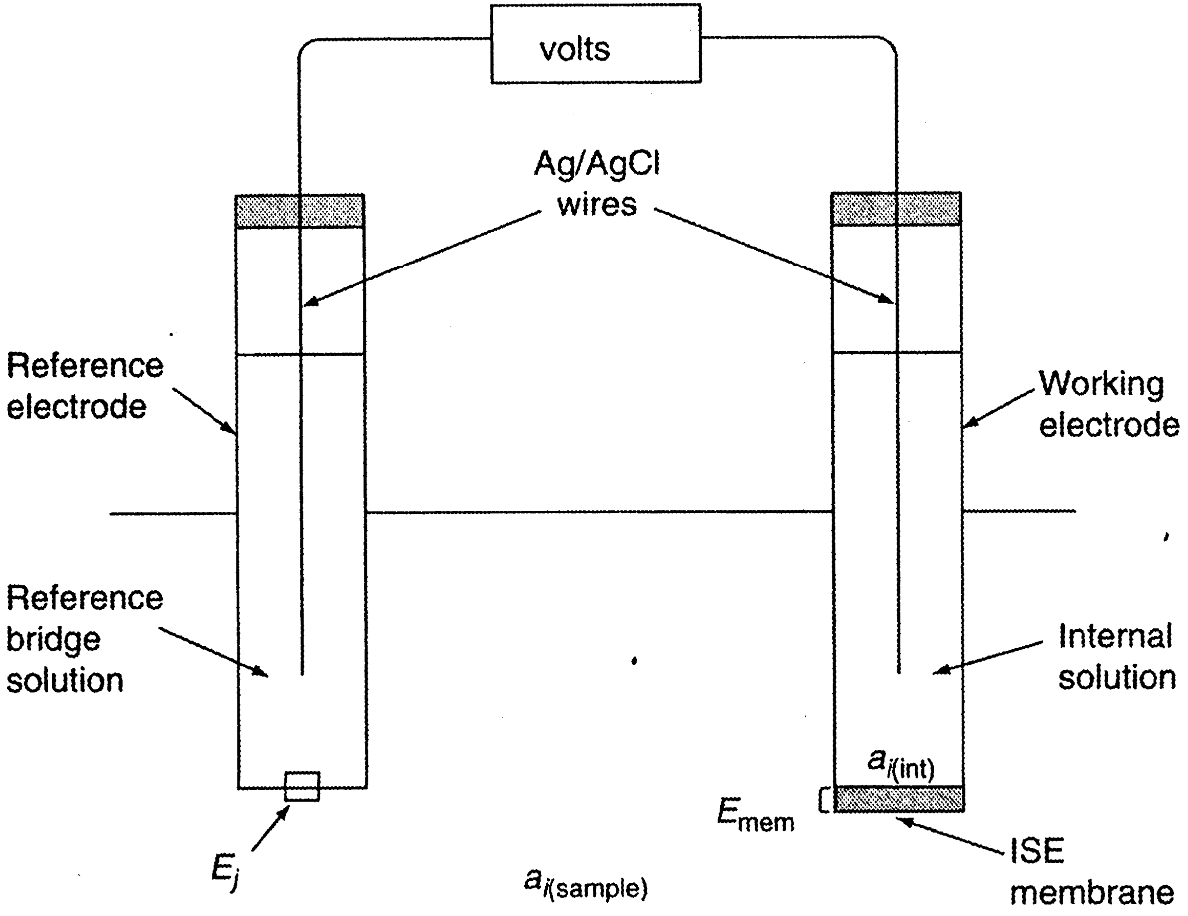
- Ion selective electrodes designed to respond to one ion more than others. Potential on the electrode is ~log(a). It’s a concentration cell with ion-selective membrane

Potentiometry

- In **potentiometry** the information on the composition of the sample is obtained through the potential appearing between the electrodes
- Equipment required:
  - ion-selective electrode,
  - reference electrode
  - potential measurement device (high-impedance mV-meter)

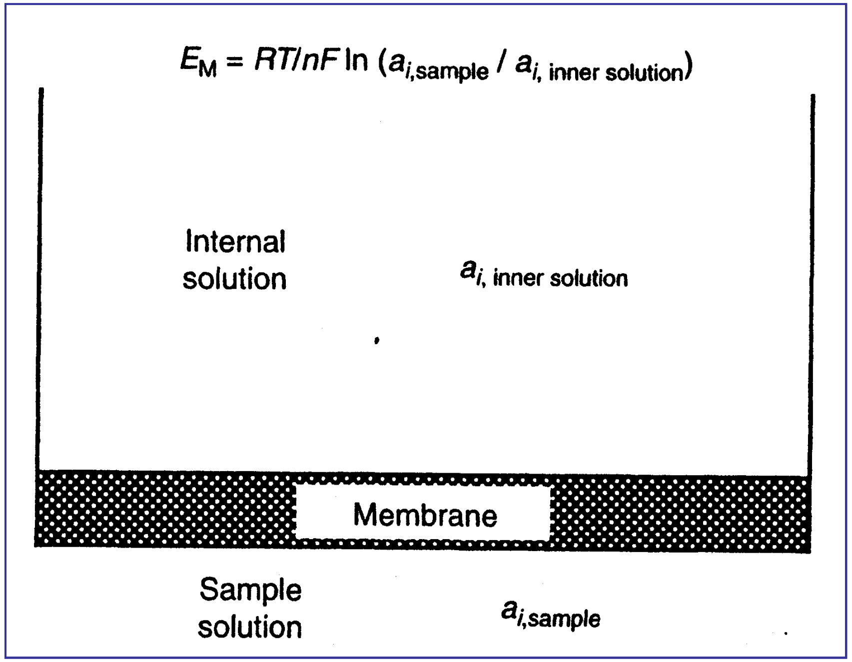
Potentiometry

- Potentiometric setup



ISE: the membrane potential

- Membrane:
  - non-porous
  - water insoluble
  - designed to yield potential primarily due to ion of interest



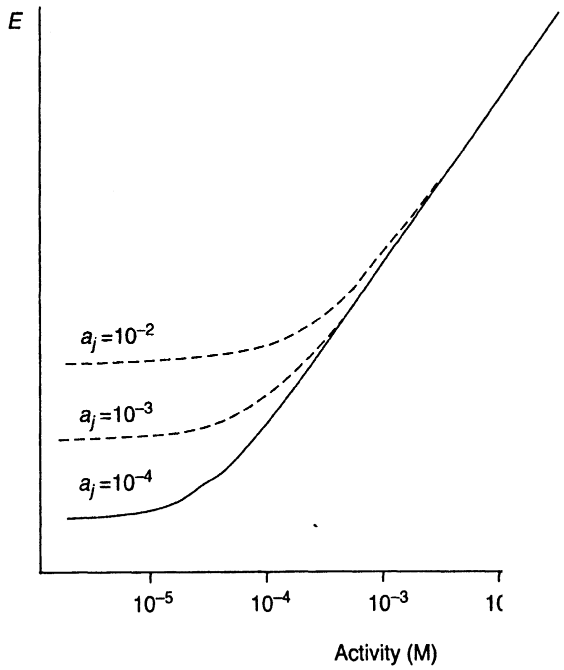
- The origin of membrane potential

- Ideally, the response of ISE: $E = K + 2.303 \dfrac{RT}{z_i F} \log a_i$

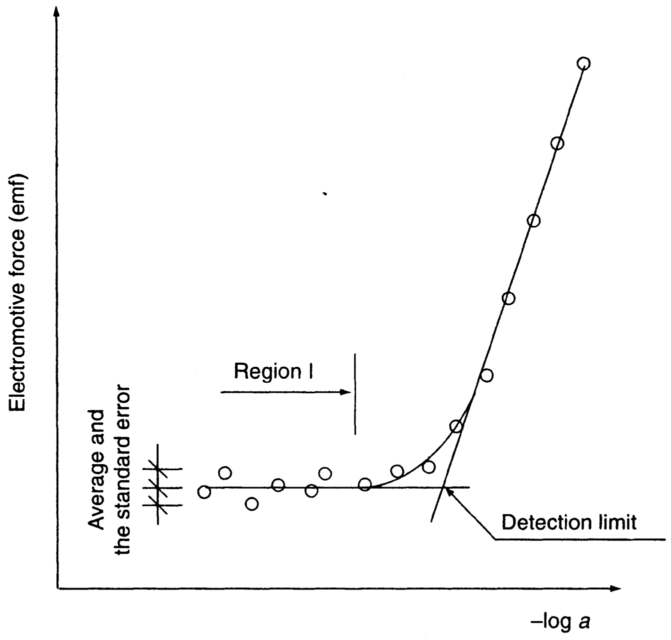
Nernstian behaviour: 59.1/z_i mV per 10-fold change in activity at 298K

Potential response of ISE

Effect of the interfering ions



Detection limit of ISE



Nikolskii-Eisenman equation

- Nearly all ISE are subject to interference from similar ions

Nikolskii-Eisenman equation:

$$E = K + \underbrace{2.303\frac{RT}{z_i F}}_{59.1/z_i\ \mathrm{mV}}\log\left(a_i + \underbrace{k_{i,j}}_{\textbf{Selectivity coefficient}} a_j^{\,z_i/z_j}\right)$$

a_i, z_i – activity and charge of primary analyte

a_j, z_j – activity and charge of interfering analyte

Determining the selectivity coefficients

Selectivity coefficients depend on the design of the electrode, primarily membrane composition and therefore should be determined experimentally for every particular set of conditions

- fixed interference (two-point mixed solution):
  - measurement on two solutions, one with a known constant concentration of interfering ion and with a zero concentration

$$E_1 = K + S\log(a_i)$$

$$E_2 = K + S\log(a_i + k_{i,j}a_j^{z_i/z_j}) \quad \Rightarrow \quad k_{i,j} = \frac{a_i 10^{(E_2-E_1)/S} - a_i'}{a_j^{z_i/z_j}}$$

- separate solution
  - calibration curve is prepared for each ion

Measurements and Calibration

- Calibration graphs
  - Standard addition

    Two measurements are made: with unknown concentration and after addition of known volume of known concentration

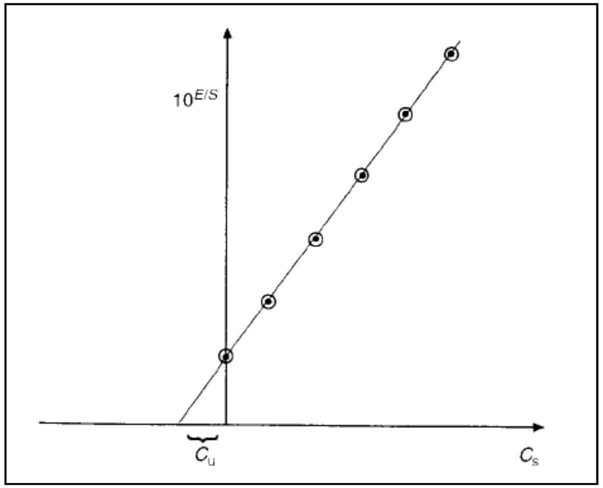
$$E_2 = K + S \log (C_u V_u + C_s V_s)/(V_u + V_s)$$

$$E_1 = K + S \log C_u$$

$$\Longrightarrow \quad C_u = C_s/\{10^{E/S}[1 + (V_u/V_s)] - V_u/V_s\}$$

  - Grant plot

    Multiple standard addition



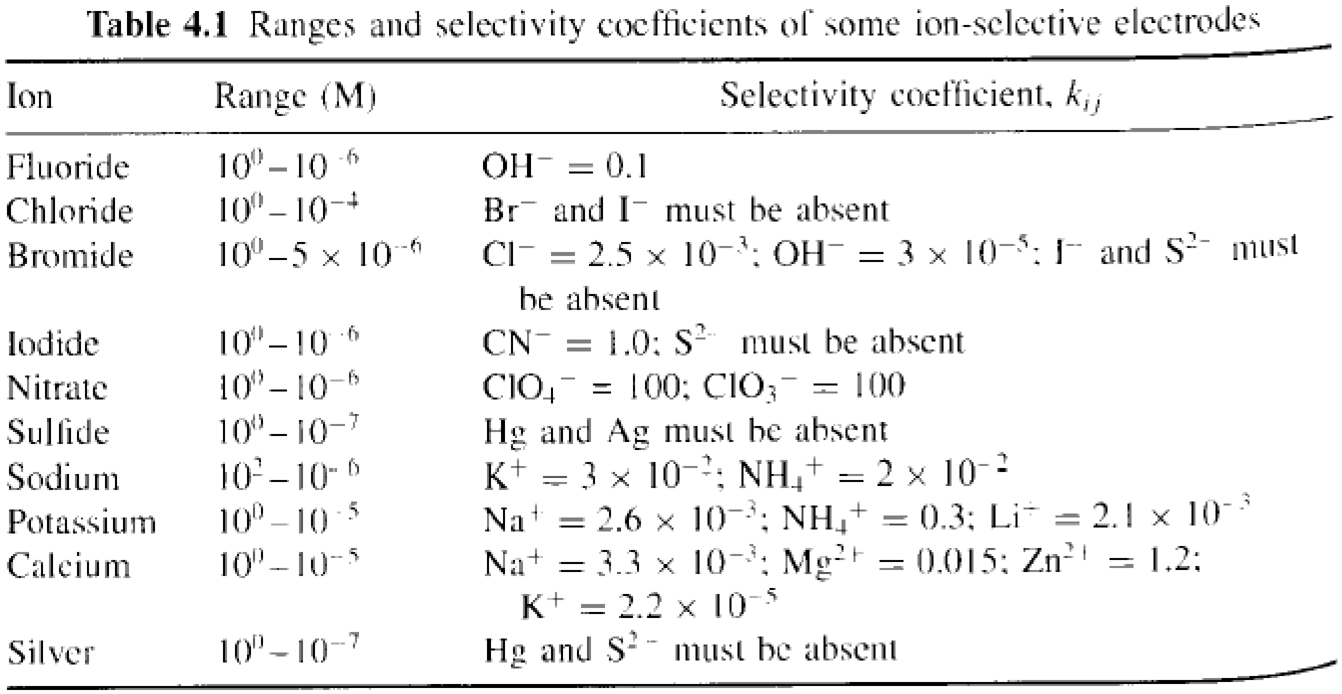


Table 4.1 Ranges and selectivity coefficients of some ion-selective electrodes

Ion	Range (M)	Selectivity coefficient, k_{ij}
Fluoride	$10^0 - 10^{-6}$	$OH^- = 0.1$
Chloride	$10^0 - 10^{-4}$	Br^- and I^- must be absent
Bromide	$10^0 - 5 \times 10^{-6}$	$Cl^- = 2.5 \times 10^{-3}$; $OH^- = 3 \times 10^{-5}$; I^- and S^{2-} must be absent
Iodide	$10^0 - 10^{-6}$	$CN^- = 1.0$; S^{2-} must be absent
Nitrate	$10^0 - 10^{-6}$	$ClO_4^- = 100$; $ClO_3^- = 100$
Sulfide	$10^0 - 10^{-7}$	Hg and Ag must be absent
Sodium	$10^2 - 10^{-6}$	$K^+ = 3 \times 10^{-2}$; $NH_4^+ = 2 \times 10^{-2}$
Potassium	$10^0 - 10^{-5}$	$Na^+ = 2.6 \times 10^{-3}$; $NH_4^+ = 0.3$; $Li^+ = 2.1 \times 10^{-3}$
Calcium	$10^0 - 10^{-5}$	$Na^+ = 3.3 \times 10^{-3}$; $Mg^{2+} = 0.015$; $Zn^{2+} = 1.2$; $K^+ = 2.2 \times 10^{-5}$
Silver	$10^0 - 10^{-7}$	Hg and S^{2-} must be absent

Rules for minimizing interference:

- adjust ionic strength of all solutions to be the same
- check for interfering ions and eventual complexing and reaction ions/agents
  e.g. In case of Fluoride ISE, pH has to be adjusted towards lower values (acid range), Al or ferric ions have to be removed with stronger complexing agent (like citric acid)

Practical aspects of ISE

- Ionic strength needs to be kept constant from one sample to the next e.g. by adding high concentration of indifferent electrolyte
- pH needs to be controlled
- Other components might be added to minimize interfering ions

$\}$ specially designed adjusters (ISA) or buffers (TISABs) are used

Potentiometric sensors

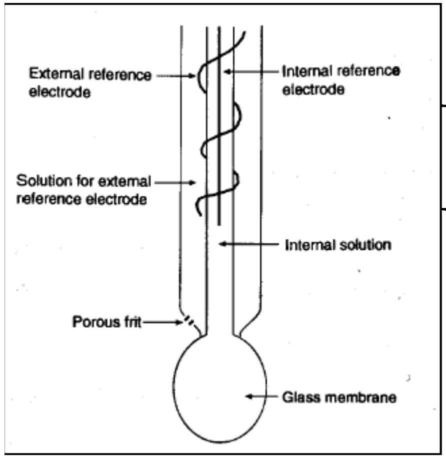
- Mainly Ion Selective electrodes

Main points:

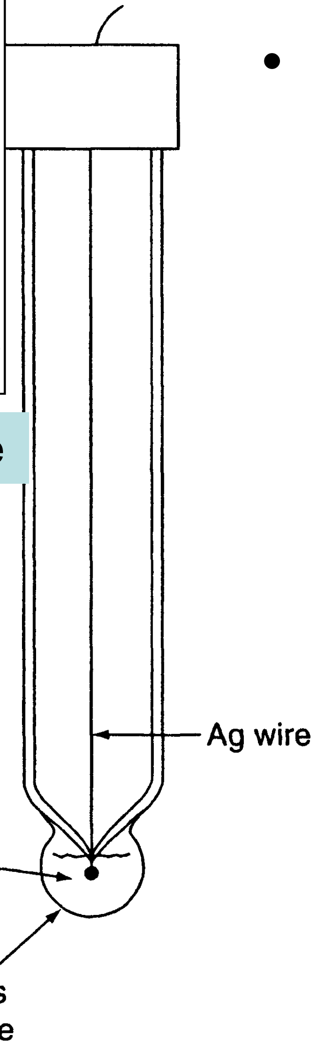
- Slope of calibration is Nernstian if S=59.1/z mV
- Electrode should be conditioned for 1-2h in the 0.01M solution of the ion of interest
- Linear range is usually between 10^{-5}M and 10^{-1}M. Below 10^{-5}M curvature due to interfering ions
- Usual criterion of stability is that E vary less than 0.1mV within 60s
- Effect of intefering ions can be described by Nicholskii-Eisenmann equation

Glass ISE

- Glass electrodes are responsive to univalent ions ((pH, Na+, Li+, K+, Ag+), the selectivity is achieved by varying the glass composition



combination electrode



- pH Glass electrodes
  - first reported in 1906 by Cremer, the commercial production started in 1940s
  - Glass contains a 3D silicate network (typically 72% SiO_2, 22% Na_2O, 6%CaO)

$$E = K + \frac{RT}{F}\ln\frac{\left[H^+\right]_{inner}}{\left[H^+\right]_{outer}} = K' + 59mV \cdot pH$$

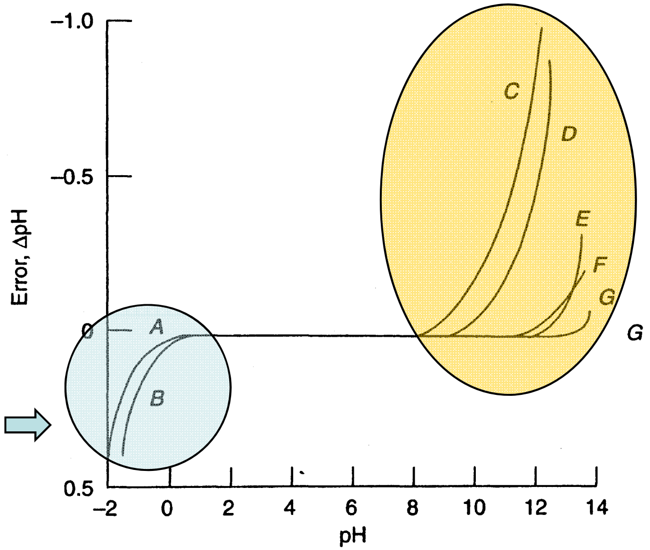
  - The selective response is related to partial exchange:

$$Na^+{}_{\text{glass}} + H_3O^+{}_{\text{soln}} \rightleftarrows Na^+{}_{\text{soln}} + H_3O^+{}_{\text{glass}}$$

Glass ISE

- Alkaline error: demonstration of lower pH at pH>11 due to interfereng effect of ions, in particular Na^+.

$$E = K + 0.059\frac{RT}{F}\log\left(\left[H_3O^+\right] + k_{H,Na^+}\left[Na^+\right]\right)$$



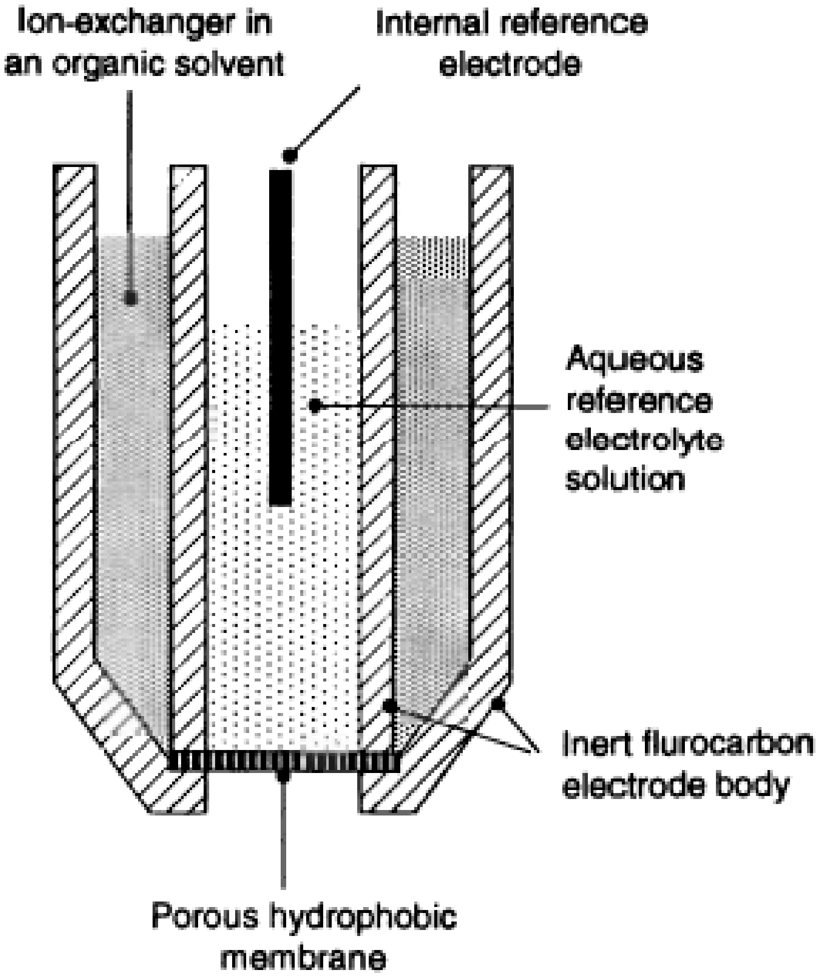
Alkaline error

Acidic error

Figure 5.7 The alkaline and acid errors of several glass pH electrodes: *A*, Corning 015/H_2SO_4; *B*, Corning 015/HCl; *C*, Corning 015/1 M Na^+; *D*, Beckman-GP/1 M Na^+; *E*, L&N BlackDot/1 M Na^+; *F*, Beckman E/1 M Na^+; *G*, Ross electrode. (Reproduced with permission from Ref. 17.)

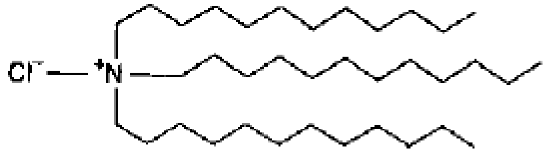
- The effect can be reduced with new glasses using Li^+ salts, lowering $k_{H,Na}$~10^{-10}.
- Many glass electrodes show acidic error: higher pH readings at pH<0.5

Liquid Ion-exchange membrane
(NO_3^-, Cu_2^+, Ca^{2+}, Cl^-,BF_4^-, K^+)



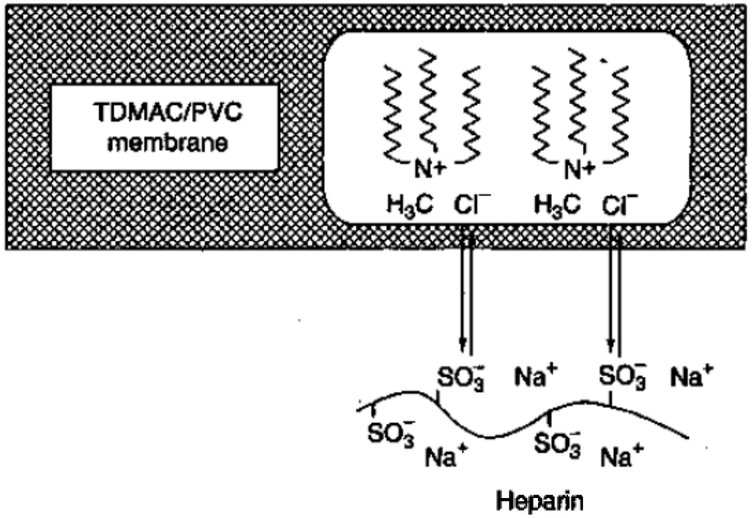
- Example: Ca^{2+} sensor
  - Liquid cation exchanger: aliphatic diester of phosporic acid $(RO)_2PO_2^-$ with R groups in C_8-C_{16} range
  - Ca^{2+} activity in the pH range of 5.5-11 can be measured down to 10^{-7}M
  - selectivity coefficients $K_{Ca,Mg}$=0.02, $K_{Ca,\ K}$=0.001.

Liquid Ion-exchange membrane
(NO_3^-, Cu_2^+, Ca^{2+}, Cl^-,BF_4^-, K^+)



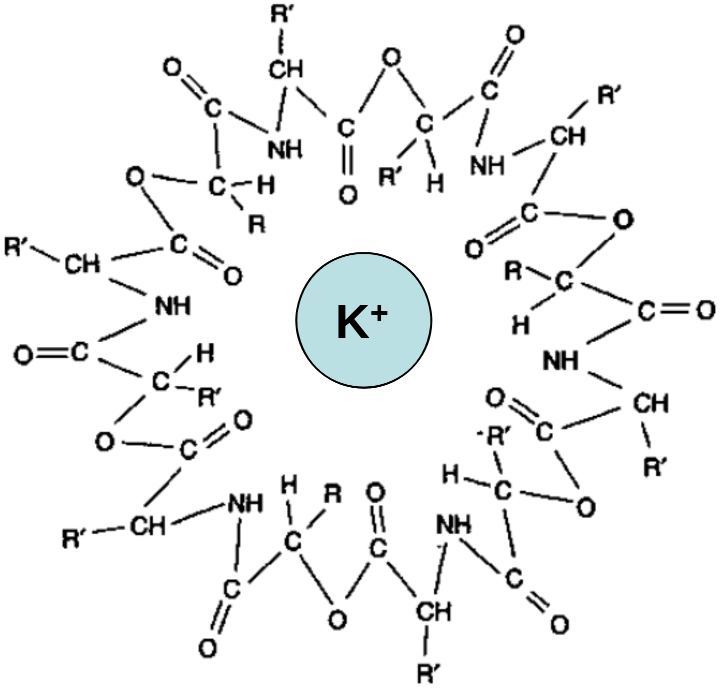
- Anion exchanger (e.g. alkyl ammonium chloride) can be used to sense for anions
- The selectivity is low and related to the anion partition into the membrane that follows Hofmeister series

large
lipophilic $> ClO_4^- > IO_4^- > SCN^- > I^- > NO_3^- > Br^- > Cl^- > HCO_3^- > H_2PO_4^-$
anions



- Very successful sensor was designed using Ammonium Tridodecylchloride (TDMAC) for detection of heparine
- Similar approach can be used for detection of other polyanions.

Liquid Ion-exchange membrane
(NO_3^-, Cu_2^+, Ca^{2+}, Cl^-,BF_4^-, K^+)



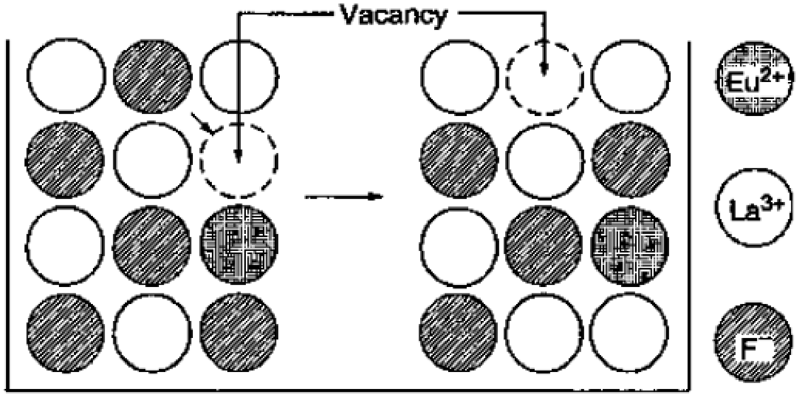
valinomycin

- Neutral carriers (e.g. crown compounds) can selectively envelop an ion in their pocket
- Binding determined by the “best fit” mechanism, very selective. E.g. valinomycin has selectivity K^+ over Na^+ ~30000.
- ionophores can be designed for both cations and anions

Solid-State membrane (Ag^+, Cl^-, Br^-,S^{2-}, SCN^- , F^+)

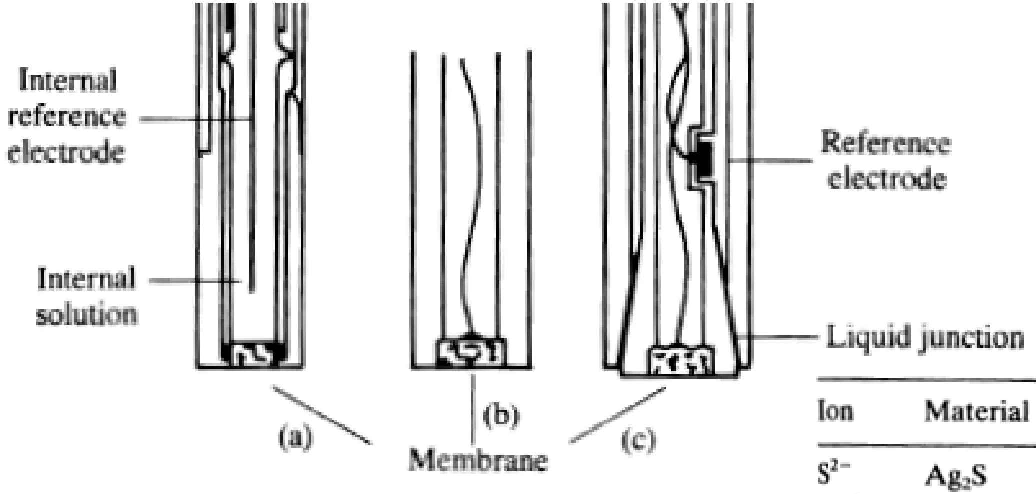
Fluor ions detection:

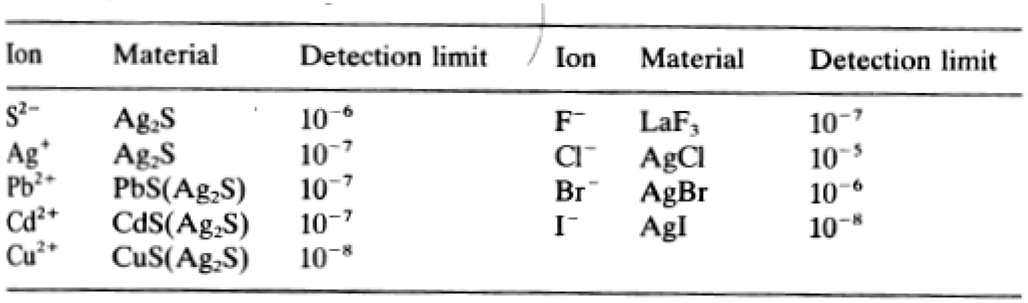
LaF_3 doped with EuF_2 to provide F vacancies



Ag | AgCl | KCl(0.1 M) ¦¦ test solution containing Ag^+ but not X^- | AgX membrane | Ag

ohmic contact





Ion	Material	Detection limit	Ion	Material	Detection limit
S^{2-}	Ag_2S	10^{-6}	F^-	LaF_3	10^{-7}
Ag^+	Ag_2S	10^{-7}	Cl^-	$AgCl$	10^{-5}
Pb^{2+}	$PbS(Ag_2S)$	10^{-7}	Br^-	$AgBr$	10^{-6}
Cd^{2+}	$CdS(Ag_2S)$	10^{-7}	I^-	AgI	10^{-8}
Cu^{2+}	$CuS(Ag_2S)$	10^{-8}			

Gas-Sensing Electrodes

- Include gas-permeable membrane and solution gas can form a buffer with
- Mainly based on pH electrodes, but other types (S^{2-}, I^-, CO_2)

$$NH_4Cl = NH_4^+ + Cl^-$$

$$NH_3 + H^+ = NH_4^+$$

$$K = \frac{[NH_3][H^+]}{[NH_4^+]};\ \log[NH_3] = pH + pK_a + \log[NH_4^+]$$

const

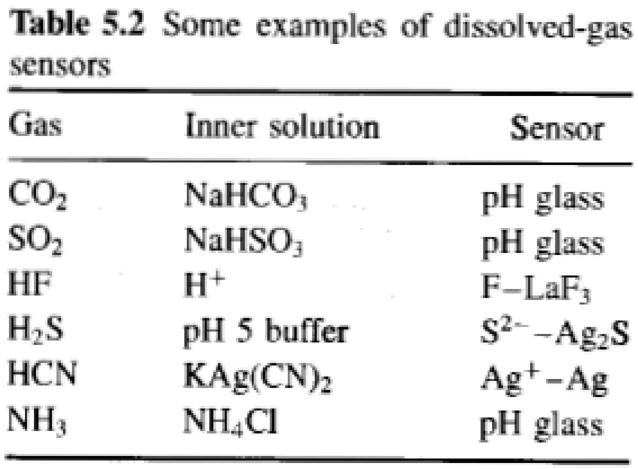
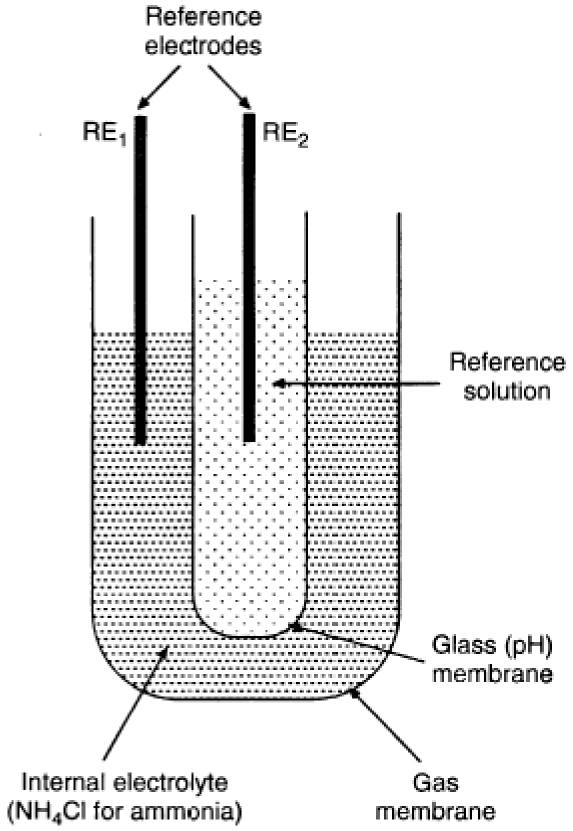


Table 5.2 Some examples of dissolved-gas sensors

Gas	Inner solution	Sensor
CO_2	$NaHCO_3$	pH glass
SO_2	$NaHSO_3$	pH glass
HF	H^+	$F-LaF_3$
H_2S	pH 5 buffer	$S^{2-}-Ag_2S$
HCN	$KAg(CN)_2$	Ag^+-Ag
NH_3	NH_4Cl	pH glass



Potentiometric Biosensors: pH linked

- Penicillin $\text{penicillin} \longrightarrow \text{penicilloate} + H^+$
- Glucose $\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$
- Urea $CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^{2-}$

Potentiometric Biosensors: Ammonia linked

Urea (in alkaline solution liberated ammonia can be detected. The sensitivity up to 10^{-6}M)

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^{+} + CO_3^{2-}$$

- Creatinine

$$\text{creatinine} \xrightarrow{\text{creatinase}} NH_3 + \text{creatine}$$

- Phenylalanine

$$\text{L-phenylalanine} \xrightarrow{\text{phenylalanine ammonia–lyase}} NH_3 + \textit{trans}\text{-cinnamate}$$

- Adenosine

$$\text{adenosine} \xrightarrow{\text{adenine–deaminase}} NH_3 + \text{inosine}$$

- Aspartame

$$\text{aspartame} \xrightarrow{\text{L-aspartase}} NH_3 + C_6H_5CH_2CH(CO_2H)NHCOCHCHCO_2H$$

Potentiometric Biosensors: CO_2 linked

- Urea
  (in acidic solution)

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^{+} + CO_3^{2-}$$

- Oxalate

$$\text{oxalate} \xrightarrow{\text{oxalate decarboxylase}} CO_2 + \text{formate}$$

$$\text{oxalate} \xrightarrow{\text{oxalate oxidase}} 2CO_2 + H_2O_2$$

- Digoxin (digoxin on PS beads labeled with peroxidase labelled anti-digoxin)

$$H_2O_2 + \text{pyrogallol} \xrightarrow{\text{peroxidase}} CO_2$$

Potentiometric sensors: Iodide selective

- Iodide selective
  - Glucose

peroxide is used to oxidize iodide in the presence of peroxidase (PO)

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + 2I^- + 2H^+ \xrightarrow{\text{PO}} I_2 + 2H_2O$$

  - Phenylalanine
    (suffer more interference than ammonia based sensor)

$$\text{L-phenylalanine} \xrightarrow{\text{LAO/PO}} H_2O_2$$

$$H_2O_2 + 2I^- + 2H^+ \longrightarrow I_2 + 2H_2O$$

Potentiometric sensors: AgS linked

- Cysteine

$$\mathrm{Ag}^{+} + x\mathrm{R}-\mathrm{S}^{y-} \longrightarrow \mathrm{Ag}(\mathrm{R}-\mathrm{S})_{x}^{(1-xy)}$$

Not very selective

or

$$\text{cysteine} + \mathrm{CN}^{-} \xrightarrow{\beta\text{-cyanoalanine synthesase}} \mathrm{HS}^{-} + \beta\text{-cyanoalanine}$$

(more specific, but cyanide interferes with electrode)

Problems

- **Atkins 7.16a:** Use the Debye–Hückel limiting law and the Nernst equation to estimate the potential of the cell Ag|AgBr(s)|KBr(aq, 0.050 mol kg^{-1})||Cd(NO$_3$)$_2$(aq, 0.010 mol kg^{-1})|Cd at 25°C.

- For Ca ISE was found that:
  S=29.6 mV, E=-20.1mV in 1mM CaCl$_2$;
  E=-19.8mV in 1mM CaCl$_2$ + 100mM NaCl
  Calculate selectivity coefficient.