

11. Biochemistry-1

Electrochemical Measurements of Biochemical Quantities

- *Biochemical Quantities*
- *Electrochemistry*
- *pH Measurement*
- *pCO₂ Measurement*
- *pO₂ Measurement*

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Chemical Quantities in Blood

Living cells are chemical factories where the input is metabolic food and the output is waste products. The functional status of an organ system can be evaluated by measuring the chemical input and output analytes of the cells.

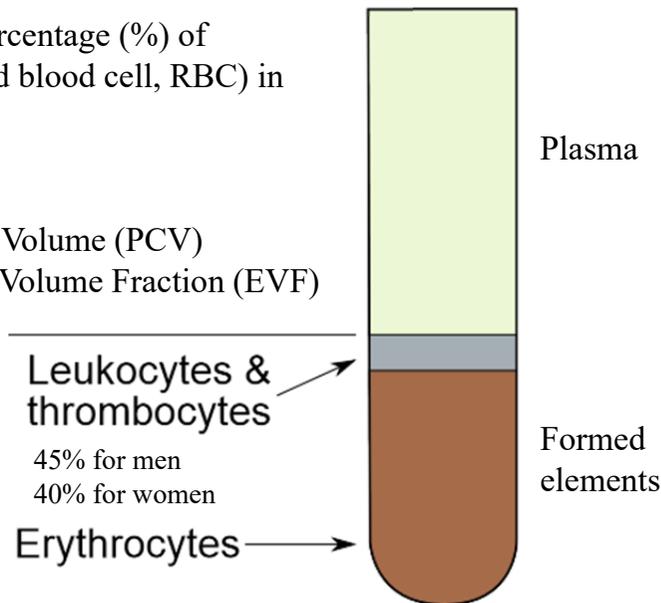
Blood Gases and Related Parameters		Electrolytes		Metabolites	
pO ₂	80–104 mm Hg	Na ⁺	135–155 mmol/l	Glucose	70–110 mg/100 ml
pCO ₂	33–48 mm Hg	K ⁺	3.6–5.5 mmol/l	Lactate	3–7 mg/100 ml
pH	7.31–7.45	Ca ²⁺	1.14–1.31 mmol/l	Creatinine	0.9–1.4 mg/100 ml
Hematocrit	40–54%	Cl ⁻	98–109 mmol/l	Urea	8–26 mg/100 ml
Total hemoglobin	13–18 g/100 ml				
O ₂ -saturation	95–100%				

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Hematocrit

The volume percentage (%) of erythrocyte (red blood cell, RBC) in blood.

- Ht
- HCT
- Packed Cell Volume (PCV)
- Erythrocyte Volume Fraction (EVF)

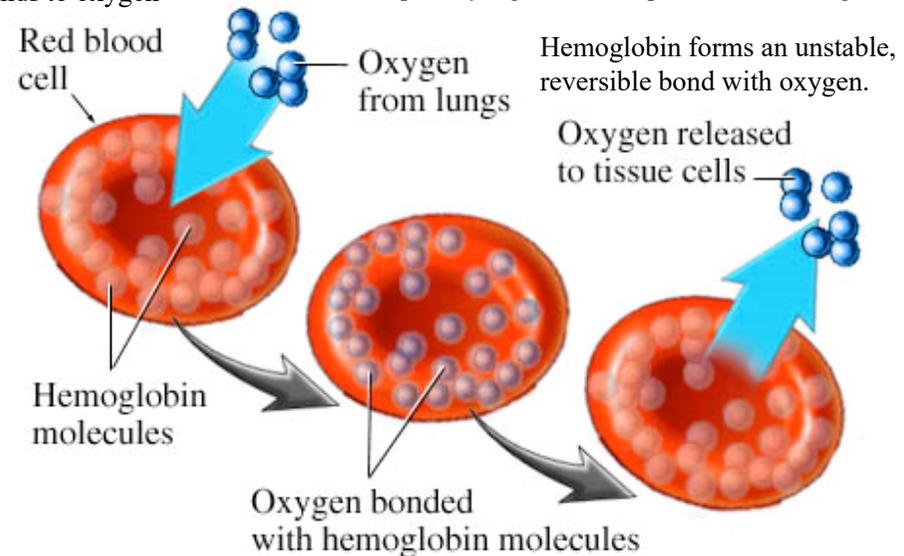


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Hemoglobin

a protein molecule that binds to oxygen

- To carry O₂ from the lung to the tissues
- To release O₂ to burn nutrients to provide energy to power the functions of the organism
- To collect the resultant CO₂ to bring it back to the respiratory organs to be dispensed from the organism

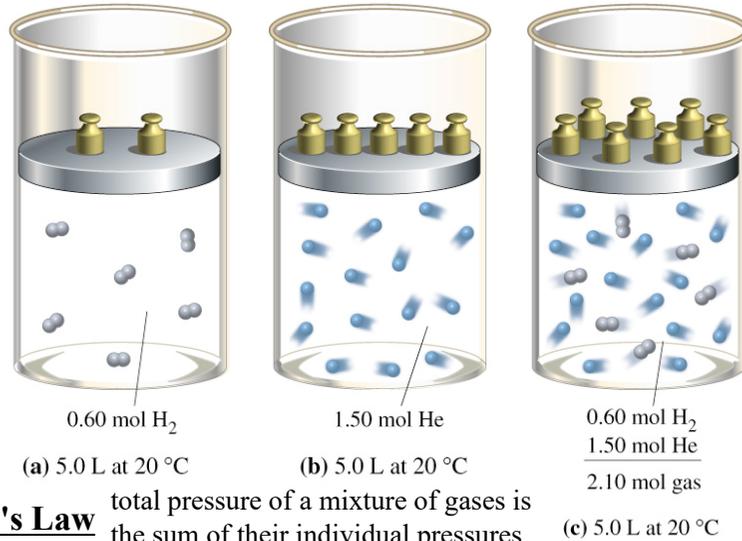


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Partial Pressures

A **gas mixture** behaves in exactly the same fashion as a **pure gas**

$$P_{H_2} = 2.9 \text{ atm} + P_{He} = 7.2 \text{ atm} = P_{\text{total}} = 10.1 \text{ atm}$$

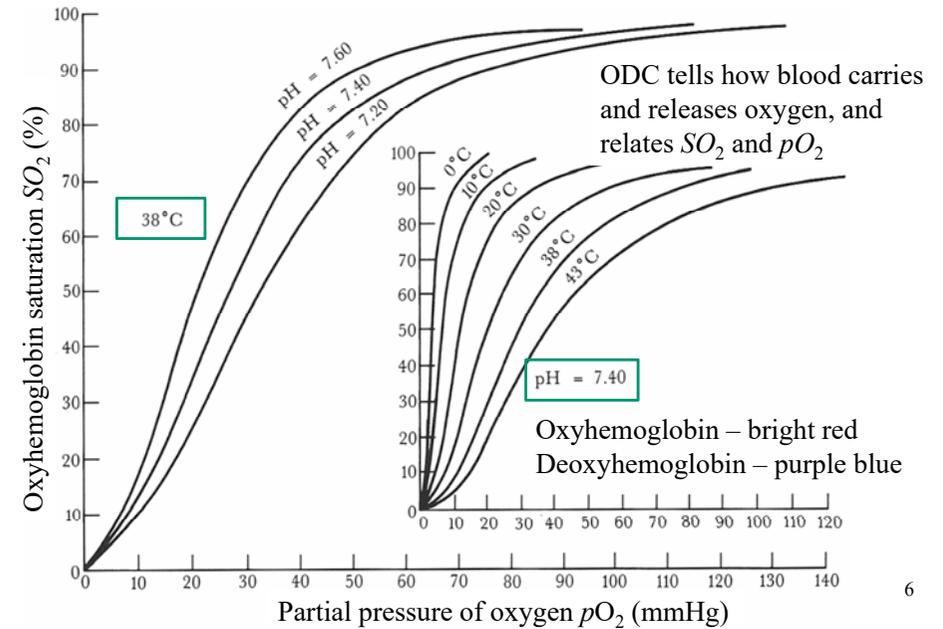


Dalton's Law

total pressure of a mixture of gases is the sum of their individual pressures

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Oxyhemoglobin Dissociation Curve



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Significance of Blood Gases

- The fast and accurate measurements of the blood levels of the partial pressure of oxygen pO_2 , the partial pressure of carbon dioxide pCO_2 , and the concentration of hydrogen ions pH are vital in the diagnosis and treatment of many pathological conditions.

Example	pCO_2 mmHg	pH	pO_2 mmHg	Interpretation	Likely causes
1	40 ± 3	7.40 ± 0.03	90 ± 5	Normal blood gas	
2	44 ± 3	7.37 ± 0.03	88 ± 5	Normal blood gas while asleep	
3	22	7.57	106	Hyperventilation	Anxiety
4	68	7.10	58	Hypoventilation	Central nervous system depression; blockage of upper airway
5	58	7.21	39	Hypoventilation and hypoxemia	Pneumonia; small-airway obstruction; severe asthma
6	61	6.99	29	Combined respiratory and metabolic acidosis and hypoxemia	Birth asphyxia; near-drowning
7	60	7.37	106	Chronic respiratory acidosis with metabolic compensation; patient is receiving supplemental oxygen	Patient has chronic lung disease and is on oxygen
8	29	7.31	106	Metabolic acidosis with respiratory compensation	Diabetic; ketoacidosis; dehydration

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Electrochemical Sensors

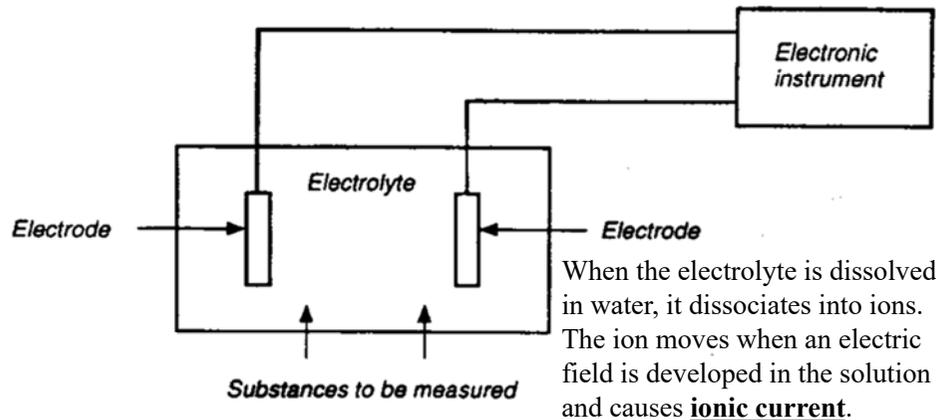
- Convert chemical quantity into electrical potential or current by using electrochemical principles
- Two electrodes + electrolyte solution + instrument
- Electrolyte is dissolved in water ⇒ ions
- Ions move in electric field in the solution ⇒ ionic current
- Ionic current ⇒ electronic current
- Electrode ⇒ interface between electrolyte and electric conductor
- Concentration of a substance to be measured is reflected in the potential between the electrodes (potentiometric sensor), or current through the electrodes (amperometric sensor)

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Basic Electrochemical System

Ionic current is measured by a pair of electrode which forms an interface between the electrolyte and the electric conductor to converts an ionic current into an electronic one.

The concentration of the substance to be measured is reflected in the potential between the electrodes, or current through the electrodes.

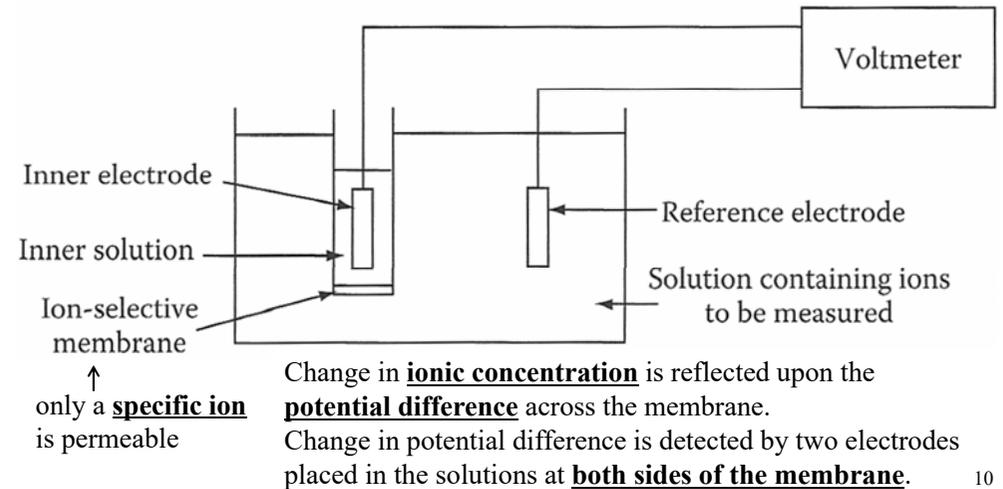


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Ion-Selective Electrode (ISE)

Potentiometric Sensor

The object quantity is reflected in the potential difference



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Amperometric Sensor

D, α : diffusion coefficient and the solubility of oxygen in the membrane

p : partial pressure of the oxygen

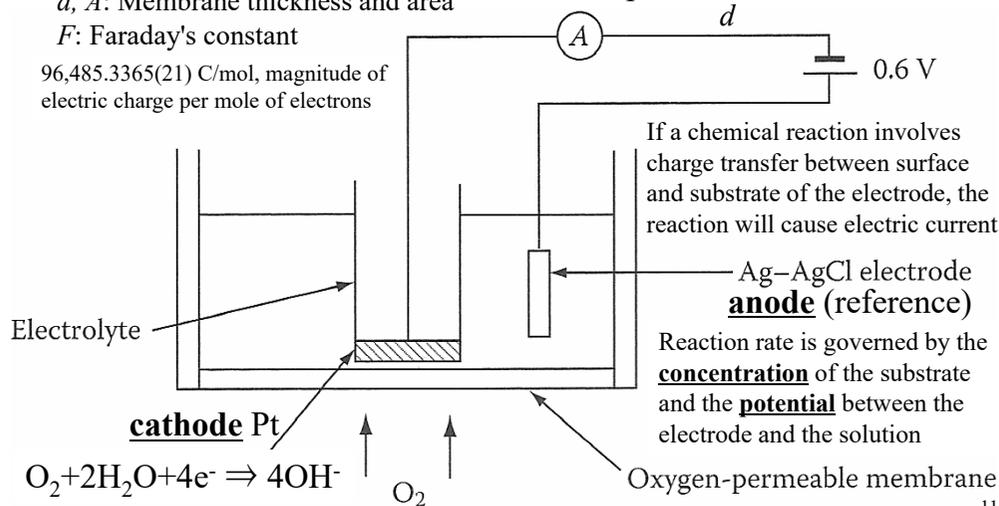
d, A : Membrane thickness and area

F : Faraday's constant

96,485.3365(21) C/mol, magnitude of electric charge per mole of electrons

If oxygen is supplied by diffusion through a membrane, the current is

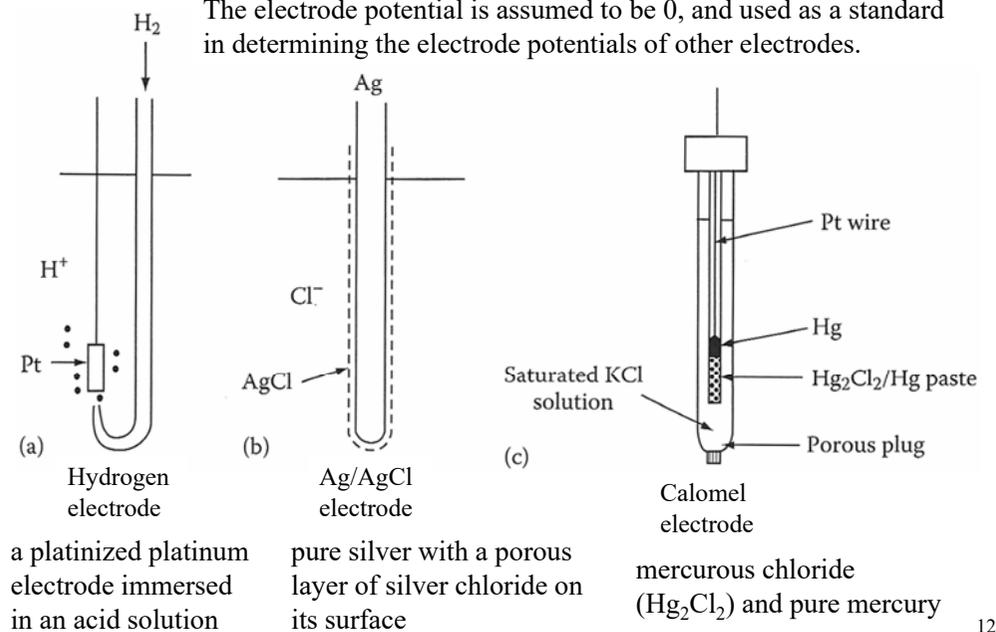
$$I = \frac{4FAD\alpha p}{d}$$



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Reference Electrode

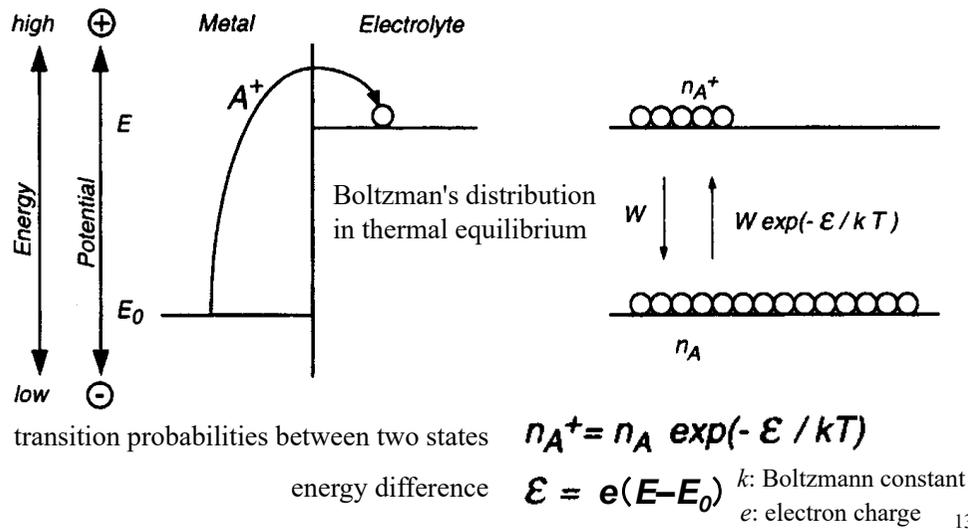
The electrode potential is assumed to be 0, and used as a standard in determining the electrode potentials of other electrodes.



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Energy Level and Transition

If a potential difference between the metal and the electrolyte exists, the redox reaction corresponds to a move of a charge between two states at different potential levels, it will accompany a gain or loss of electrical potential energy.



Nernst Equation

- At **thermal equilibrium**, the transition rates on both directions between two energy levels are equal so that:

$$n_{A^+} = n_A \cdot \exp\left(\frac{-\epsilon}{kT}\right)$$

- Substituting ϵ equation and replacing populations of atoms in both states by respective activity a_A and a_{A^+}

$$a_{A^+} = a_A \cdot \exp\left(\frac{-e(E - E_0)}{kT}\right)$$

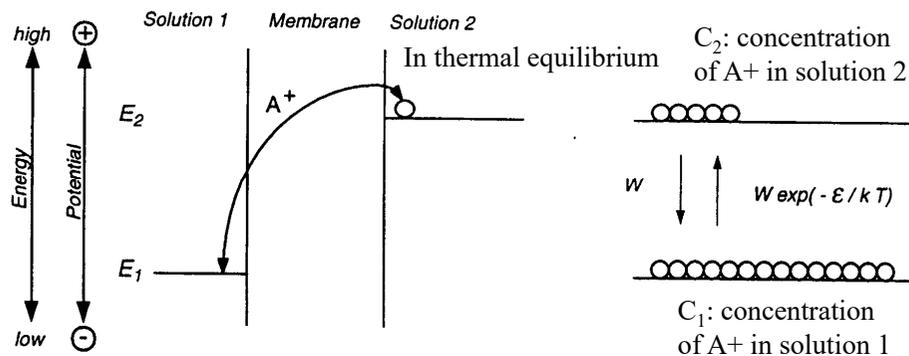
- Thus, E becomes

$$E = E_0 + \left(\frac{kT}{e}\right) \ln\left(\frac{a_{A^+}}{a_A}\right)$$

Membrane Potential

A membrane is permeable to ion A^+ , but impermeable to other ions, and is placed between solutions 1 and 2.

E_1 and E_2 are potentials of solution 1 and 2, ion A^+ will gain energy $c=e(E_2 - E_1)$ when it moves from solution 1 to 2.



$$E_2 - E_1 = \text{constant} + \left(\frac{RT}{F}\right) \ln(K_A a_{A^+} + K_B a_{B^+} + K_C a_{C^+} + \dots)$$

K_A : selectivity coefficient, a_{A^+} : activity of A^+

Electrolyte Solution

- Dissociation and association occur as $AB \leftrightarrow A^+ + B^-$
- When both direction reactions are balanced in equilibrium, the degree can be described as

$$\frac{a_{A^+} \cdot a_{B^-}}{a_{AB}} = K \quad \frac{[A^+][B^-]}{[AB]} = k$$

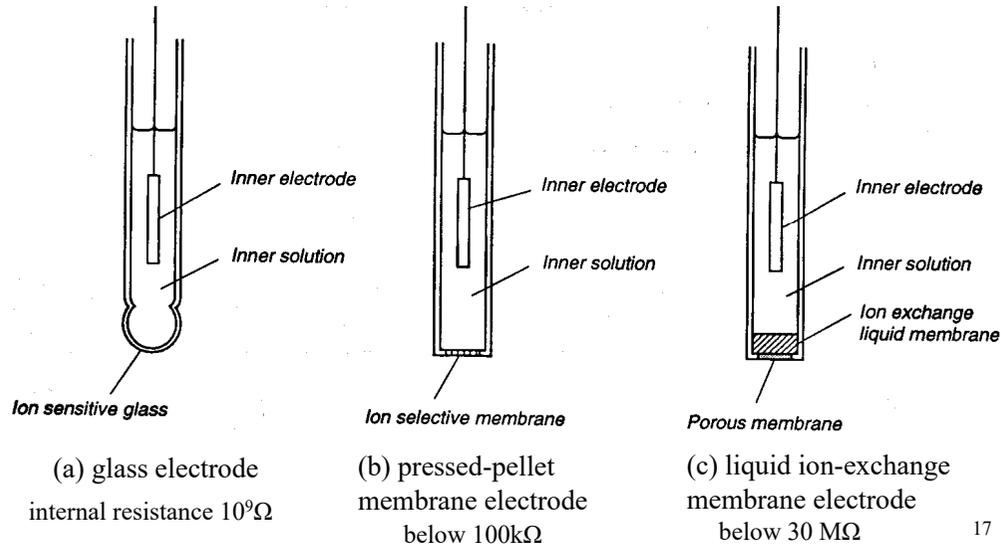
where $[A^+]$, $[B^-]$ and $[AB]$ are molar concentrations of A^+ , B^- and AB , respectively

a_{A^+} , a_{B^-} and a_{AB} are activities of A^+ , B^- and AB , respectively

- Both k and K are called equilibrium constants

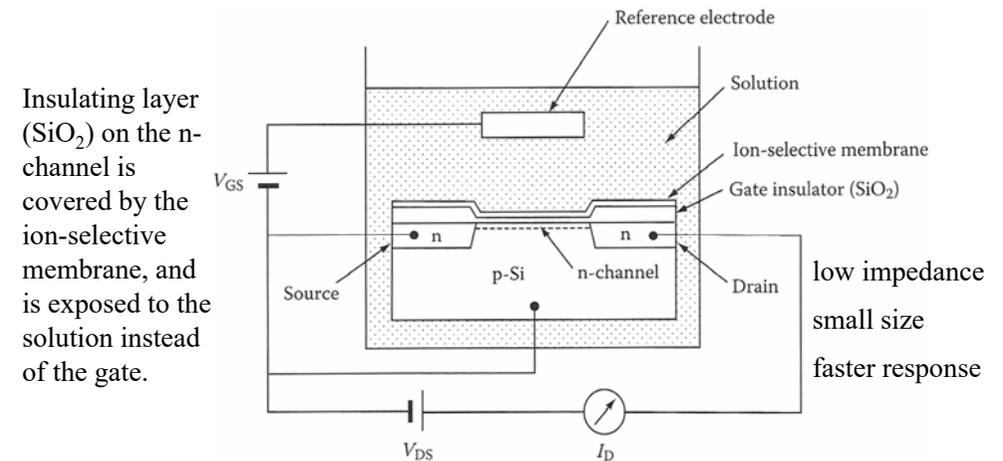
Ion Selective Electrodes

ISE properties depend on the electrical property of the ion-selective membrane. The response time of the ISE depends on the structure, size, and geometry of the electrode, and the electrical property of the membrane material (sec-min).



Ion-selective Field-effect Transistor (ISFET)

fabricated on a p-type silicon substrate similar to MOSFET



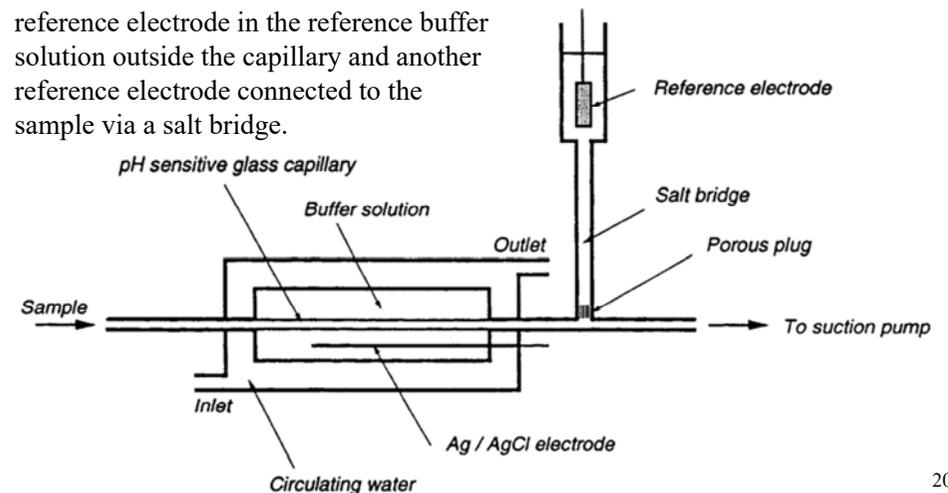
By placing a reference electrode in the solution, a bias voltage V_{GS} is applied onto the insulating layer on the conductive channel similar to applying bias voltage to an FET. The potential developed at the membrane by the existing ion in the solution is measured by the change in current through the channel. 18

pH Measurement

- The acid-base status of the blood is assessed by measuring the hydrogen ion concentration $[H^+](mol/L)$
- The ion concentration is described by $pH = -\log_{10}[H^+]$
- In a neutral solution, $pH = 7.0$
- The normal range of $pH = 7.40 \pm 0.03$
- $pH \downarrow \rightarrow$ quantity of hydrogen ions $\uparrow \rightarrow$ rate of excretion of $CO_2 \downarrow \leftarrow$ respiratory acidosis + production of fixed acid $\uparrow \leftarrow$ diabetic ketoacidosis + abnormal losses of bicarbonate (principal hydrogen ion buffer in the blood)
- $pH \uparrow \rightarrow$ quantity of hydrogen ions $\downarrow \rightarrow$ rate of excretion of $CO_2 \uparrow \leftarrow$ respiratory alkalosis + abnormal losses of acid \leftarrow prolonged vomiting = metabolic alkalosis
- pH is measured by utilizing a **glass electrode** that generates an electric potential when solutions of differing pH are placed on the two sides of its membrane.

pH Glass Capillary Electrode

The sample is introduced into the capillary. The potential built up across the glass membrane is measured between a reference electrode in the reference buffer solution outside the capillary and another reference electrode connected to the sample via a salt bridge.



pCO₂ Measurement



$$[\text{CO}_2] = a \cdot p\text{CO}_2 \quad a=0.0301 \text{ mmol/liter/mmHg}$$

$$k' = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \quad k = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2]} = \frac{[\text{H}^+][\text{HCO}_3^-]}{a \cdot p\text{CO}_2}$$

[H₂CO₃] is proportional to [CO₂]

$$\log[\text{H}^+] + \log[\text{HCO}_3^-] - \log a - \log p\text{CO}_2 - \log k = 0$$

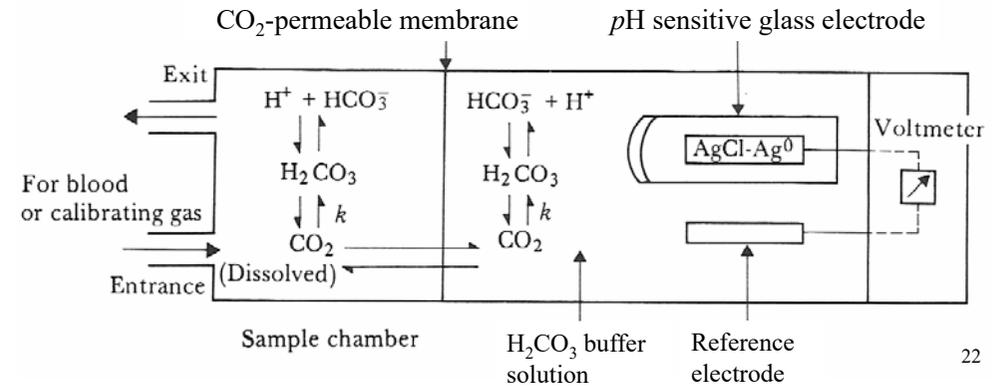
$$\text{pH} = \log[\text{HCO}_3^-] - \log a - \log p\text{CO}_2 - \log k$$

pH has a linear dependence on $-\log(p\text{CO}_2)$

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pCO₂ Electrode

- CO₂ diffuses across the membrane to establish the same concentration in both chambers. If there is a net movement of CO₂ into (or out of) the chamber containing the buffer, [H⁺] increases (or decreases), and the pH meter detects this change.
- Because pH is linear dependent on $-\log(p\text{CO}_2)$, once establishing a calibration curve of pCO₂ vs. pH, the specimen's pCO₂ can be obtained by the measured pH value from this curve.

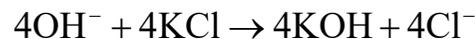


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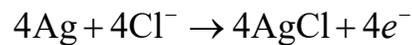
pO₂ Measurement

The presence of O₂ and the resulting chemical reaction can be thought of as producing in the circuit a variable source of current the value of which is directly proportional to the pO₂ level.

Reduction reaction at cathode



Oxidation reaction at anode



O₂ consumed in the reaction is a function of the area of Pt electrode exposed to the reaction solution and the permeability of semipermeable membrane to O₂.

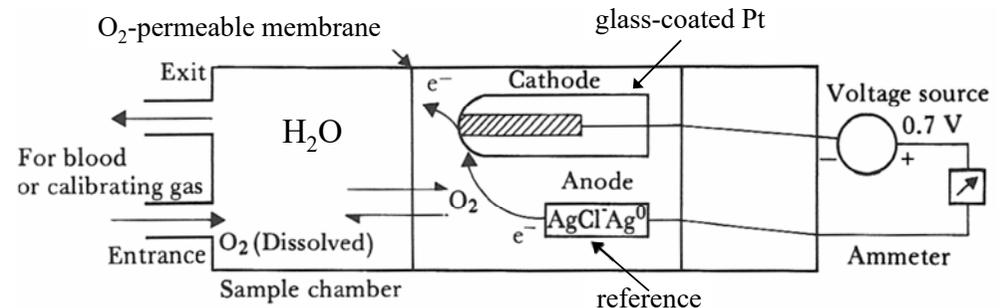
The cathode (measurement electrode) is constructed of glass-coated Pt, and the anode (reference electrode) is made of Ag/AgCl

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pO₂ Electrode

Twice calibration is done by using two gases of known O₂ concentration.

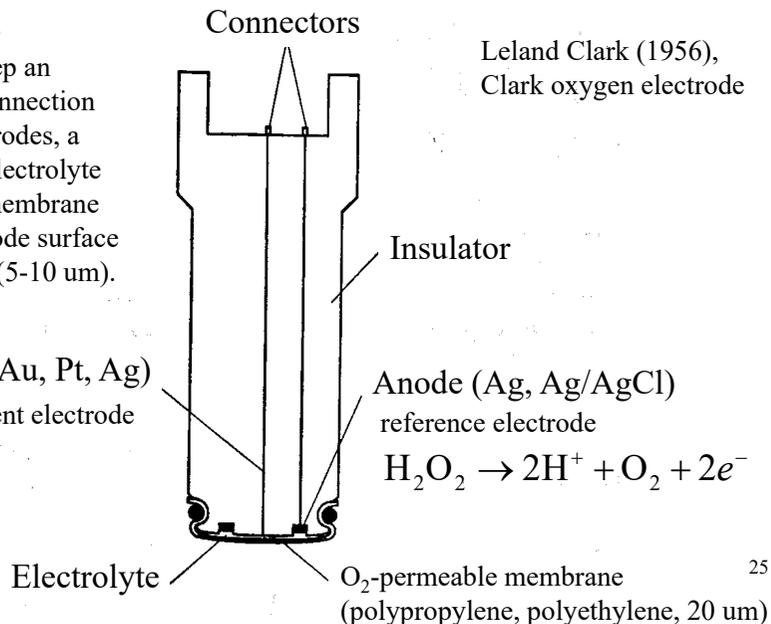
1. the gas containing no O₂ is provided. The pO₂ meter output is set to zero after equilibrium of O₂ content is achieved (90s).
2. the gas with known concentration is used to determine the second point on the pO₂-electrode-current calibration curve.



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Clark Polarographic Electrode

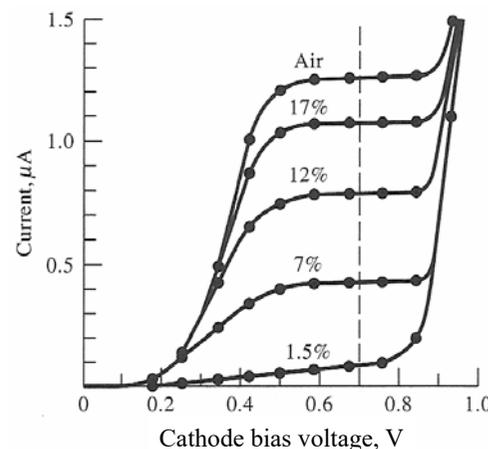
In order to keep an electrolytic connection between electrodes, a thin layer of electrolyte between the membrane and the electrode surface is maintained (5-10 μm).



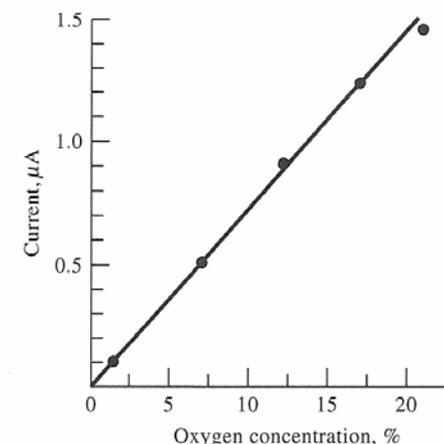
Leland Clark (1956),
Clark oxygen electrode

Polarogram of Clark Electrode

A polarizing voltage of 600 to 800 mV is supplied for the reduction-oxidation reactions to occur. The value of $p\text{O}_2$ is determined by using the right chart that indicates the current through the electrodes is proportional to $p\text{O}_2$.



(a) current vs. voltage for a $p\text{O}_2$ electrode

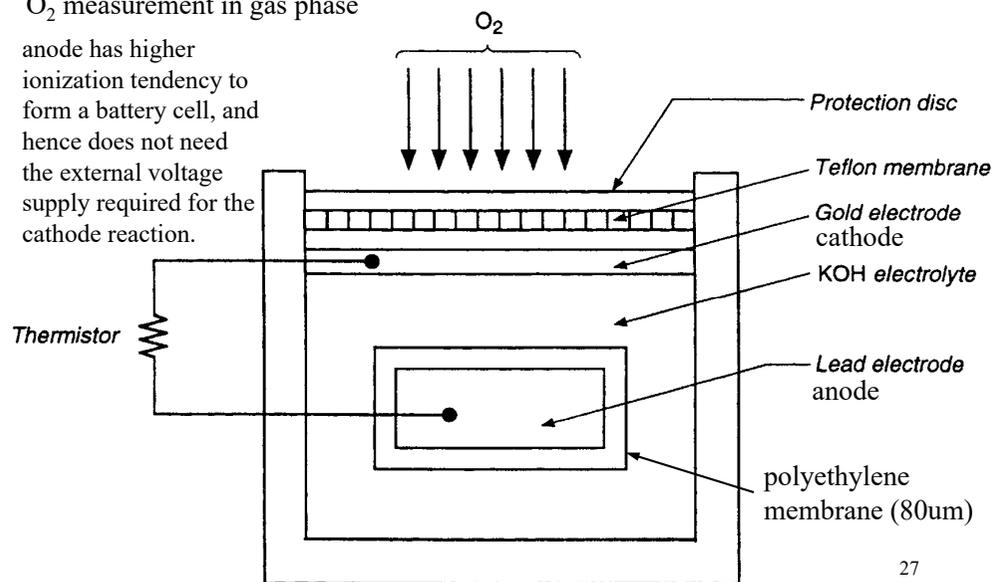


(b) current vs. $p\text{O}_2$

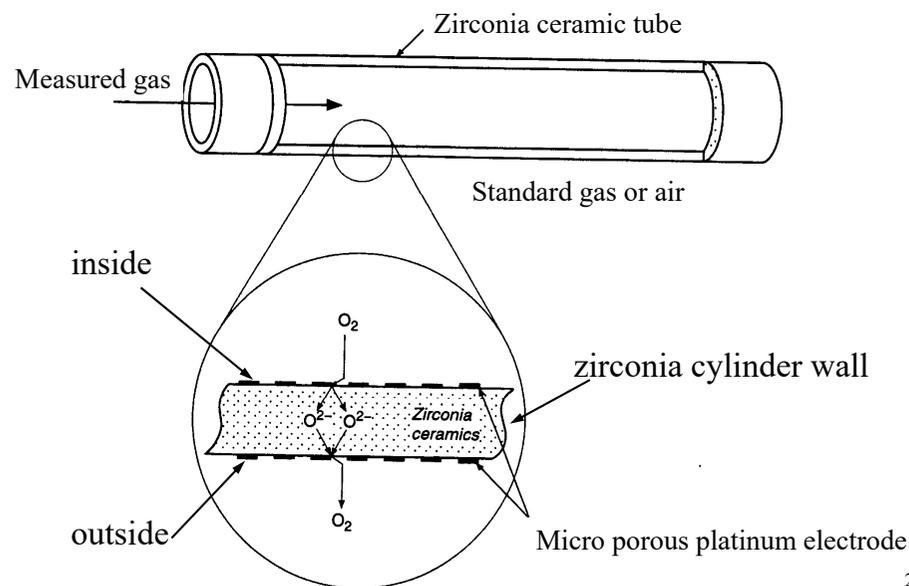
Galvanic-cell $p\text{O}_2$ Electrode

O_2 measurement in gas phase

anode has higher ionization tendency to form a battery cell, and hence does not need the external voltage supply required for the cathode reaction.

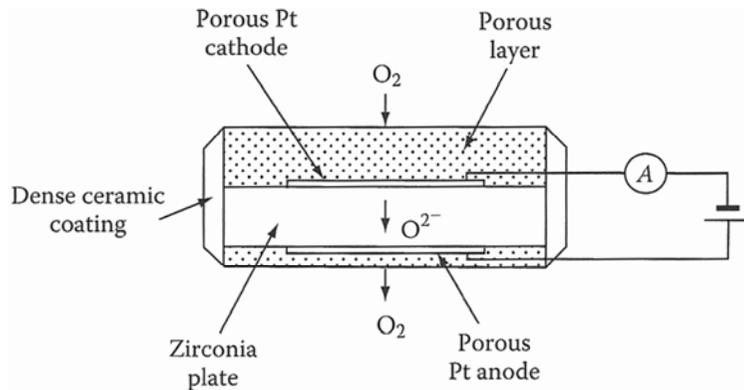


Zirconia $p\text{O}_2$ Electrode



Limiting-current pO_2 Electrode

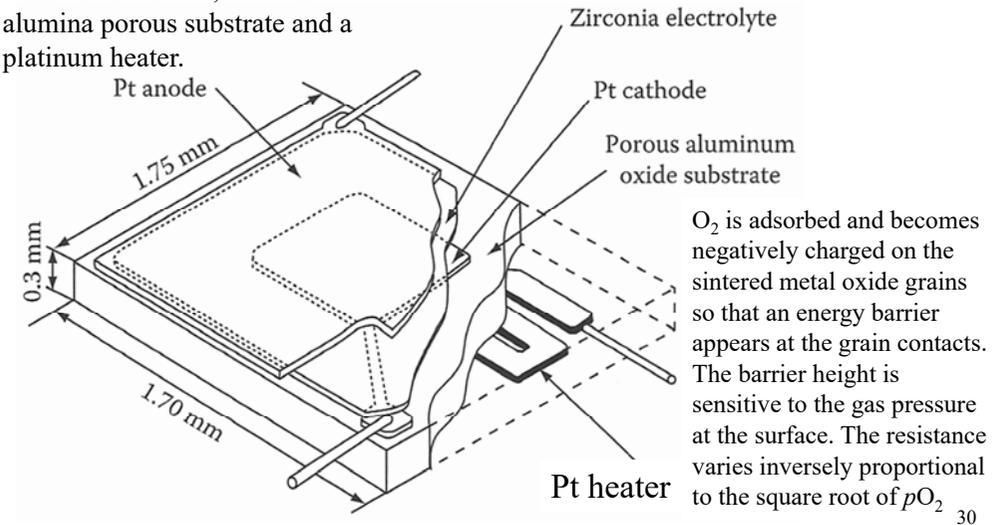
- O_2 ion with a negative charge is transported to the positive side through the negative porous layer.
- pO_2 under the porous layer becomes zero, and the current drain ceases as long as no O_2 is supplied to the electrode under the layer.
- If O_2 diffuses through the porous layer, current flows in proportion to the diffused O_2 flux.
- An current proportional to pO_2 in the surrounding space is outputted.



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Configuration of Limiting-current pO_2 Sensor

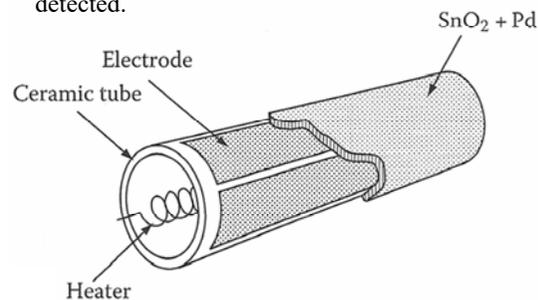
Zirconia electrolyte is sandwiched by two deposited thin platinum films as electrodes, over an alumina porous substrate and a platinum heater.



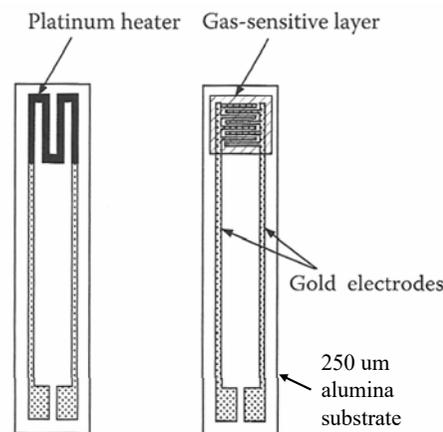
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Metal-oxide pO_2 Sensors

Gold electrodes are deposited on a small ceramic tube, and tin oxide layer is formed on it. Catalysts are added to the metal oxides so as to modify the sensitivity to a specific gas. By combining metal oxide and catalyst, different gasses such as CO, alcohols, H_2 , O_2 , H_2S , halogenated hydrocarbons, and NH_3 can be detected.



(a) Taguchi gas sensor



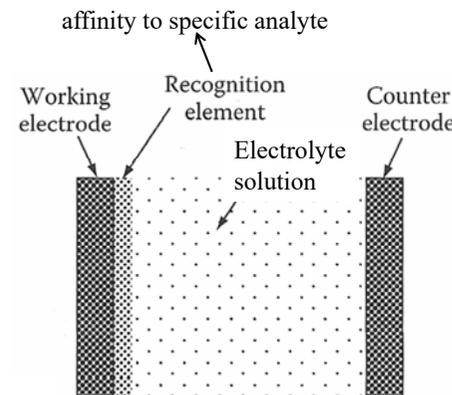
(b) Thick-film micro gas sensor

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The electric conductivity of a solution is almost proportional to ionic concentration \rightarrow salt meter

Impedimetric Electrode

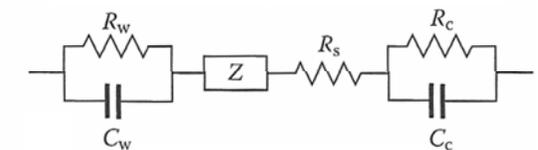
The impedance varies when the analyte binds on it. The impedance change in the recognition element is detected by measuring impedance between two electrodes.



(a) electrode structure

Resistance arises when charges move between the electrode and solution, whereas capacitance arises when charges are bounded in the layer. Both components are used for detecting analyte. When the analyte causes a change in charge transfer, it can be detected by a change in resistivity, while if the analyte causes a change in dielectric property, or a change in permittivity, it can be detected by capacitance change. When capacitive detection is used, the electrode surface is usually covered with an insulating layer so as to reduce charge transfer.

R_w, C_w : working electrode resistance and capacitance
 R_c, C_c : counter electrode resistance and capacitance
 R_s : solution resistance
 Z : recognition element impedance

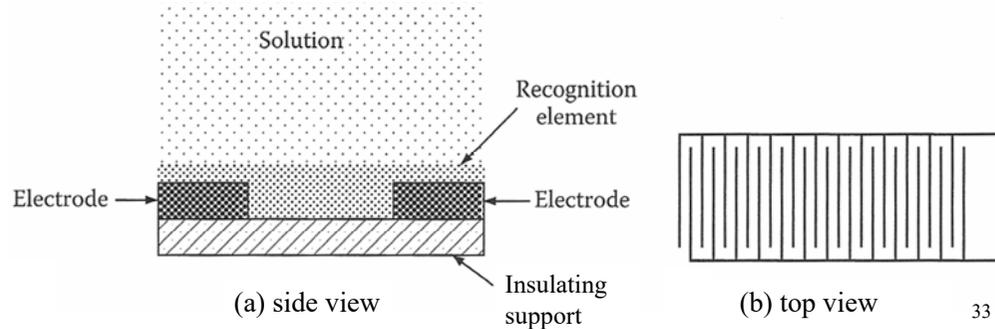


(b) equivalent circuit

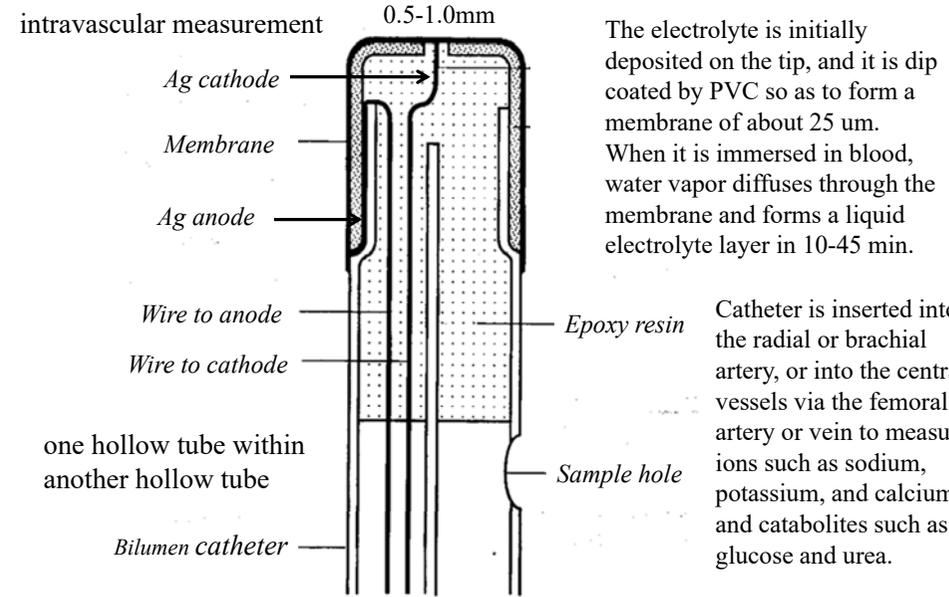
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Impedimetric Interdigitated Electrode

When a small amount of analyte is bound on the electrode surface, the resulting change in impedance is small. If the analyte is labeled by an enzyme so that the enzyme catalyzes a chemical reaction, one enzyme molecule can produce many product molecules as long as there exist substrate molecules in reasonable concentration. If the product molecule is insoluble and precipitates onto the electrode surface, the resistance and capacitance will be increased. Such a catalyzed reaction can be regarded as a chemical amplification that increases the sensitivity of impedimetric detection significantly.

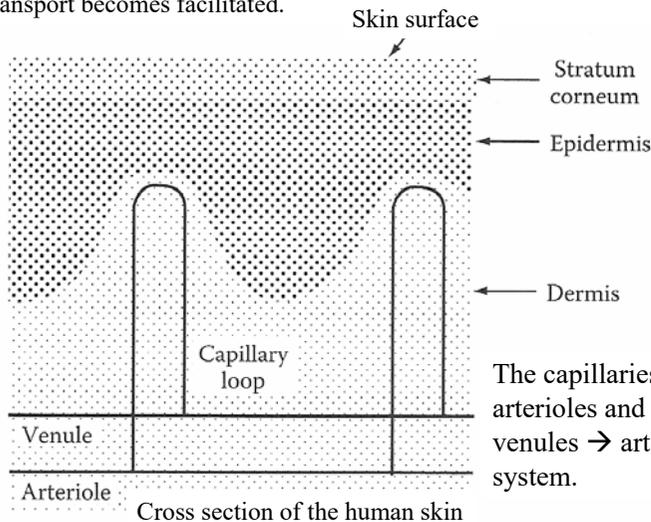


Catheter Clark pO_2 Electrode



Skin as a Membrane

Normally the skin is not very permeable to gases. At higher temperatures the skin's ability to transport gases is improved. The heat dilates the blood vessels, blood flow increases and gas transport becomes facilitated.

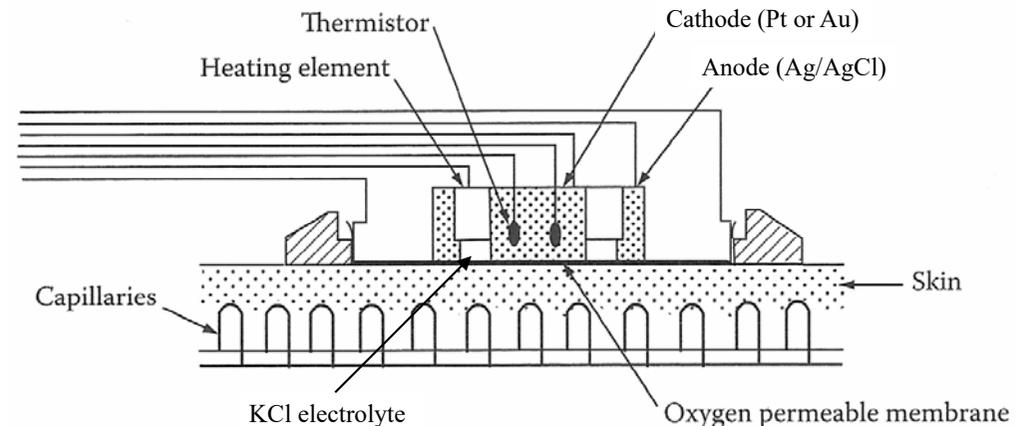


a nonliving layer of dehydrated cells that serves as a barrier. Its electrical resistance is rather high, which reduces the risk of hazardous electrical currents entering the body. a living layer consisting of proteins, lipids, and melanin-producing cells. perfused by capillaries arranged in vertical loops

The capillaries receive blood from arterioles and release blood to venules → arterial-capillary-venule system.

The vasodilatation by skin heating and the associated increased perfusion is a prerequisite for $tcpO_2$ measurements.

Transcutaneous pO_2 ($tcpO_2$) Electrode



$tcpO_2$ assesses the oxygen tension in the skin under the electrode and not in the systemic circulation, i.e. local level, not systemic level of pO_2 .

To "arterialise" capillary blood flow, skin heating to 44-45°C is necessary.

tcpO₂ & *tcpCO₂* Combined Electrode

tcpO₂ reflects the function of several physiological processes forming the links of O₂ transport chain, e.g., gas exchange in the lung, blood circulation at various levels in the circulatory system, gas exchange at the cellular level, etc.

tcpCO₂ varies inversely with alveolar ventilation and gives instant information about the effectiveness of ventilation.

