ELECTROCHEMICAL & OPTICAL BIOSENSORS

Lecture Notes

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- The cells in the body act as chemical factories whose input is metabolic food and whose output is waste products. External physical or internal cognitive stimulations can also interfere with some chemical processes.
- Therefore, it is essential for the physician to analyze the chemistry of the body.
- Important critical care analytes are the blood levels of pH, P_{O_2}, P_{CO_2}, hematocrit, total hemoglobin, O_2 saturation, electrolytes as Na^+, K^+, Ca^{++} and Cl^-, various metabolites as glucose, lactate, creatinine and urea.
- These masurements are traditionally performed in a centralized clinical laboratory which has some drawbacks as delay time (at least 30 min), errors related to the the origin of sample and to the sample handling techniques.
- Biosensor technology allows for montoring the blood chemistry of the patient in the surgical and critical care environments.
- Biosensors when combined with electronics and closed loop control systems will also allow for real time therapeutic applications as regulation of anesthesia and control of insulin secretion or drug delivery

Blood Gas and Acid Base Physiology

98% of oxygen is carried in blood as attached to hemoglobin (Hb) and 2% is dissolved in plasma.

Oxygen saturation is defined as the ratio of oxygenated Hb to total Hb as

$$S_{O_2} = \frac{[HbO_2]}{[total\ Hb]} \times 100$$

Arterial partial pressure of oxygen P_{O_2} determines the efficency of alveolar ventilation whereas S_{O_2} indicates the amount of oxygen per unit of blood. Normal range of P_{O_2} is 90-100 mmHg. Its decrease indicates either

1. decreased ventilation which is about the delivery of oxygen exchange between the inspired air and blood due to obtsructive airway problems, paralysis of ventilatory muscles, fluids filling the alveoli in pnemonia or edema.
2. decreased delivery of blood to the lungs where oxygen is supplied due to congenital cardiac problems shunting the lungs during circulation ot pulmonary obstruction caused by emboli.

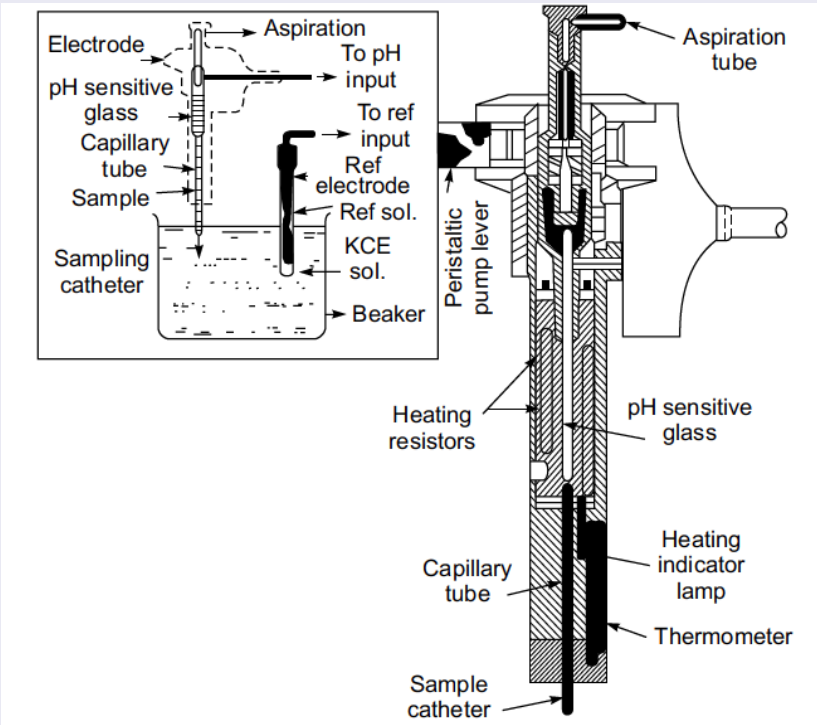
P_{CO_2} level (35-40 mm Hg normal level) is an indicator of ventilation and is increased in the first group but usually normal in the second group.
Normal acid level of blood (7.38-7.44) is indicated by $[H^+]$ and defined as

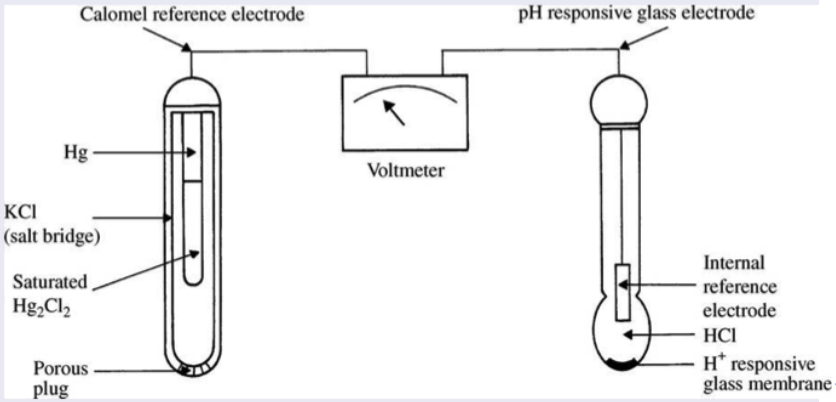
$$pH = -\log_{10}[H^+]$$

Decrease in pH or increase in $[H^+]$ indicates decreased rate of CO_2 excretion called *acidosis* whose opposite case is known as *alkalosis* .

Measurement of Blood *pH*

One glass electrode and one reference or calomel electrode are used.
Impedance of glass electrode $\sim$ 100-1000 M.
Cable must be screened and grounded to the case of measuring instrument because of ac capacitive coupling.
Temperature compensation is available.





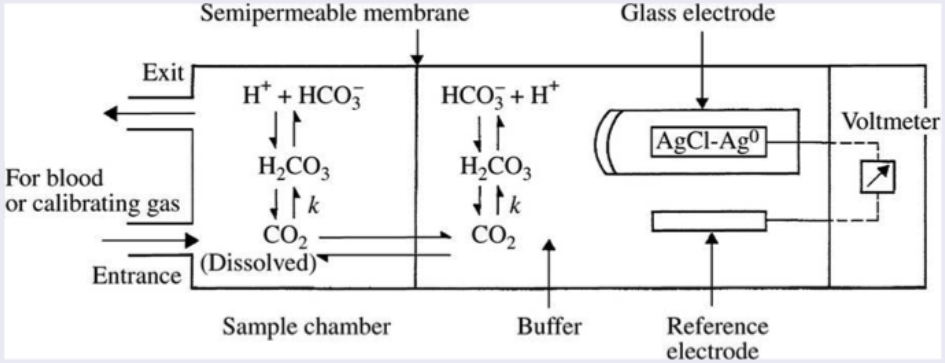
Measurement of Blood P_{CO_2}

P_{CO_2} is related to pH in blood through the relation

$$\log_{10} P_{CO_2} \approx -pH + \log_{10}[HCO_3^-] - \log_{10} k - \log_{10} a$$

where $a = \frac{[CO_2]}{P_{CO_2}} \approx 0.03 \frac{mmol/liter}{mmHg}$ and $k = \frac{[H^+][HCO_3^-]}{[CO_2]}$

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_2^-$$



Severinghaus electrode: In contrast to pH measurements, pH electode is placed in buffer solution as a reference.

The membrane passes CO_2 but not ionic particles as H^+ or HCO_3^-.

A net flow of CO_2 in either direction changes the $[H^+]$ which is detected by pH meter.

System must be calibrated by two gases of known P_{CO_2} before each use.

Self Study Question

1. A doctor submerges a pH electrode into the patient's blood sample, which has a pH of 6.9. Both electrodes are almost identical except that the glass electrode containing 10 mM HCl has a half-cell potential of 0.021 V and the reference electrode in the sample has a half-cell potential of 0.036 V. What voltage does the doctor read?

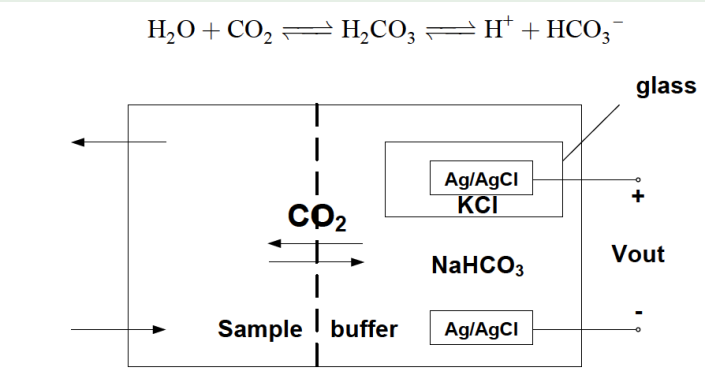
$$V_{glass} - V_{ref} = E_{glass} - E_{ref} + \frac{RT}{F}\ln(10)\underbrace{\log_{10}\frac{[H^+]_{sample}}{[H^+]_{glass}}}_{pH_{glass}-pH_{sample}}$$

$$= 0.021 - 0.036 + 62mV(2 - 6.9) = -319mV$$

2. The carbon dioxide electrode is calibrated to 40 mmHg at pH 7.4. What is the partial pressure of carbon dioxide in the patient's blood?
$\log_{10} P_{CO_2} = -pH + constant$
$\log_{10} P_{CO_{2cal}} = -pH_{cal} + constant$
$P_{CO_2} = P_{CO_{2cal}} 10^{(7.4-6.9)} = 126.5$ mmHg

Self Study Question

Consider the Severinghaus electrode shown below for measurement of P_{CO_2}. The solution internal to the glass membrane is $0.1 mol/L\ KCl$ in pure water, and the glass membrane is permeable to H^+ only. The buffer contains a saturated solution of $NaHCO_3$, and is separated from the sample by a CO_2 permeable membrane. Assume $RT/F \ln(10) = 60mV$ at room temperature.



a) You measure an output voltage V_{out} of $60mV$. What does that imply about the pH in the buffer solution?

b) The concentration of $NaHCO_3$ in the buffer solution is adjusted so that the output voltage is zero for a calibration sample of known partial pressure $P_{CO_{2cal}} = 1kPa$. Find the partial pressure P_{CO_2} of a blood sample in terms of the output voltage V_{out} measured for that sample.

c) Why is it necessary to have the blood go through the sample chamber at nonzero flow rate? How is the measurement affected when the blood sample has a fixed finite volume?

Self Study Question

a) $V_{out} = \frac{RT}{F} \ln(10) \log_{10} \frac{[H^+_{buffer}]}{[H^+_{KCl}]} = 60mV\,(pH_{KCl} - pH_{buffer}) = 60mV$

Since KCl as a pure salt is pH neutral, $pH_{KCl} = 7$.

$pH_{buffer} = pH_{KCl} - 1 = 7 - 1 = 6$

b) The concentration of $NaHCO_3$ in the buffer solution is adjusted so that the output voltage is zero for a calibration sample of known partial pressure $P_{CO_{2cal}} = 1kPa$. Find the partial pressure P_{CO_2} of a blood sample in terms of the output voltage V_{out} measured for that sample.

$\log_{10} P_{CO_2} = \frac{V_{out}}{60} + const$

$\log_{10} P_{CO_{2cal}} = \frac{0}{60} + const$

$P_{CO_2} = P_{CO_{2cal}} 10^{\frac{V_{out}}{60(mV)}}$

c) Why is it necessary to have the blood go through the sample chamber at nonzero flow rate? How is the measurement affected when the blood sample has a fixed finite volume? The sample needs to pass through the chamber at non zero flow rate because there needs to be enough supply of CO_2 in the sample so that its concentration does not get diluted by the supply already in the buffer solution. Otherwise, for a sample of limited volume, its CO_2 concentration will mix with that of a previous sample.

Measurement of Blood P_{O_2}

P_{O_2} measurement is based on a reduction on a cathode

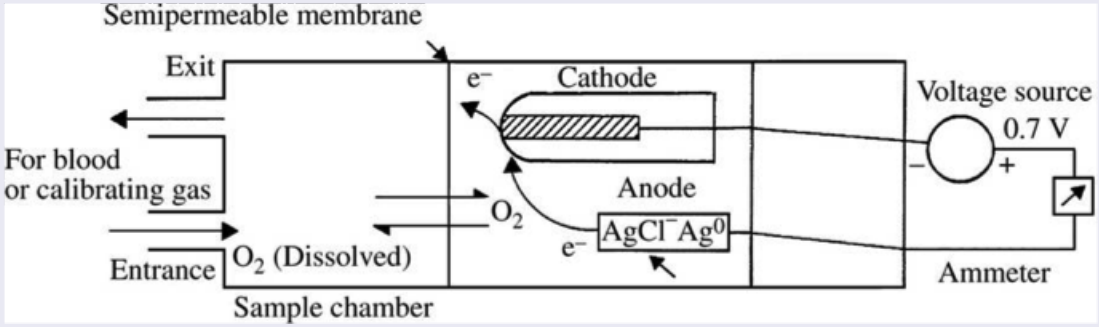
$$O_2 + 2H_2O + 4e^- \longrightarrow 2H_2O_2 + 4e^- \longrightarrow 4OH^-$$

$$4OH^- + 4KCl \longrightarrow 4KOH + 4Cl$$

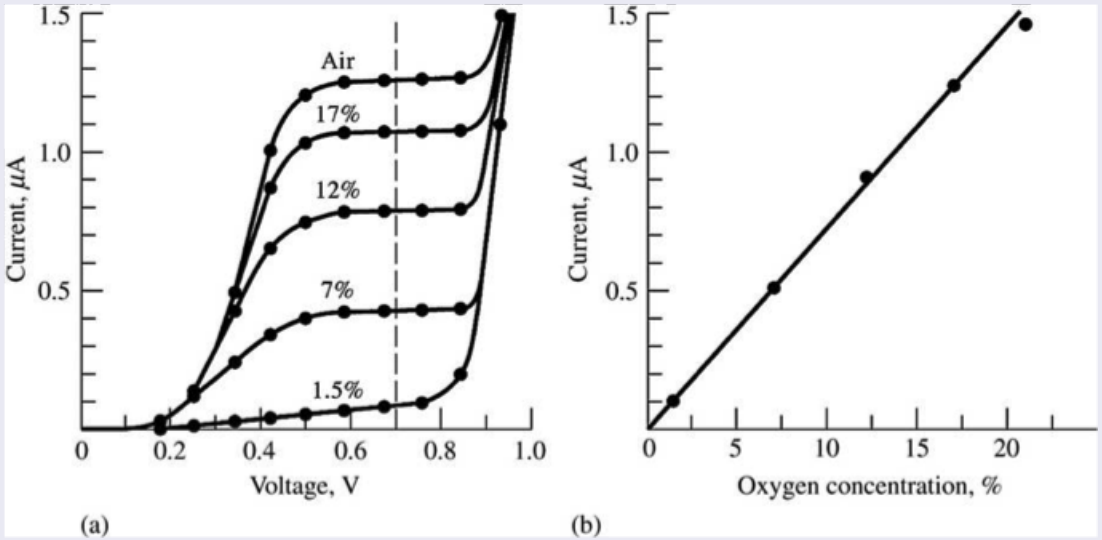
At the anode of the P_{O_2} electrode which acts as the reference electrode, oxidation occurs producing four electrons in the reaction

$$4Ag + 4Cl^- \longrightarrow 4AgCl + 4e^-$$

Oxygen from the blood diffuses across the membrane into the electrolyte filling solution and is reduced at the cathode. The circuit is completed at the anode, where silver is oxidized, and the magnitude of the resulting current indicates the partial pressure of oxygen.



Current vs polarizing voltage of P_{O_2}.



A polarizing voltage of 0.6-0.8 V gives a linear relationship between current output and % O_2.

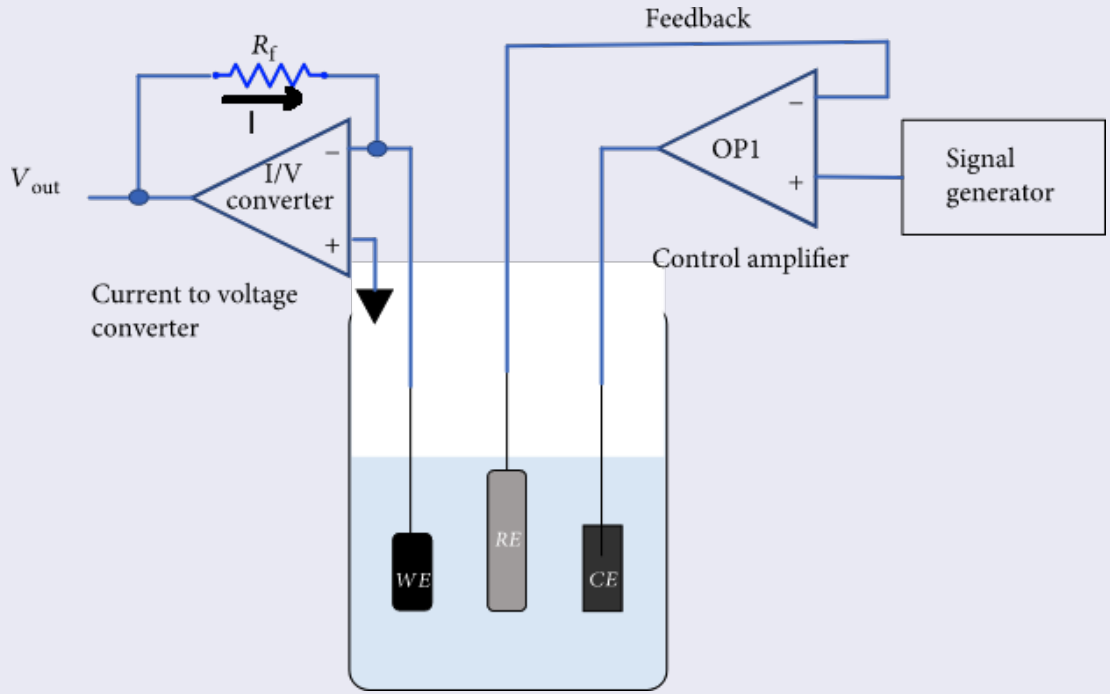
$I \approx 4F[O_2]\phi$,

ϕ : sample flow rate (l/s)

F : Faraday's constant *i.e.* $F = 1.6 \cdot 10^{-19} \cdot 6 \cdot 10^{23} = 96,485 c/mol$

Potentiostat

A circuit measuring the current through the working electrode while applying a voltage of 0.7 V across the reference and control electrodes.



$V_{out} = IR_f$:Output voltage is proportional to current (transresistance R_f)
$R_f \sim$ several $M\Omega$ for sensitivity (mV/nA)

Self Study Question

Using a potentiostat with an effective resistance of 100 MΩ, the doctor applies 0.7 V across an oxygen probe, which produces a current. What voltage signal will the doctor measure if the silver electrode is degrading at a rate of 0.3 mmol/s?

$Ag + Cl^- \rightleftharpoons AgCl + e^-$

One electron enters the potentiostat for every Ag atom consumed.

1 mole : $6.022 \cdot 10^{23} e^-$

1 coulomb : $6.241 \cdot 10^{18} e^-$

1 mole : $\frac{6.022 \cdot 10^{23}}{6.241 \cdot 10^{18}} coulomb = 96,485 = F$

$V_{pot} = 100 M I_{pot}$

$= 100M \cdot F \cdot 0.3 mmol/s = 100 \cdot 10^6 \cdot 96485 \cdot 3 \cdot 10^{-4} = 2.9 \cdot 10^9 \text{V}$

Potentiostat will be saturated by this enormous voltage level

Self Study Question

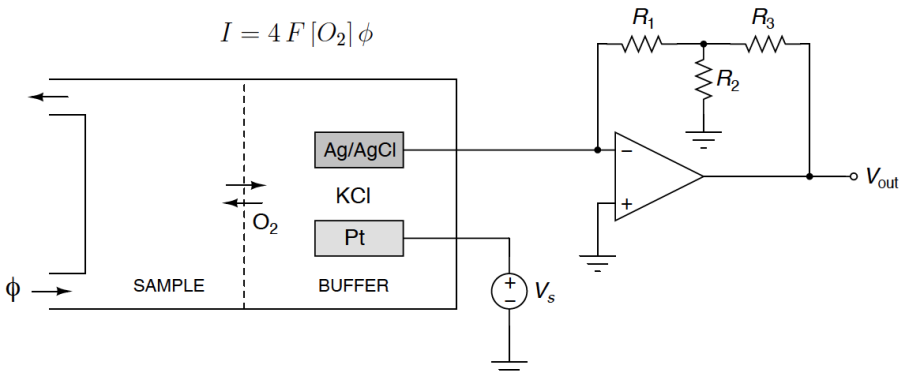
Consider the P_{O_2} sensor below consisting of a Clark electrode and a transimpedance amplifier (TIA). The flow rate ϕ of the sample through the chamber is maintained constant at $1ml/s$, the solution in the buffer is $0.1mol/l\ KCl$, and the values of TIA resistances are $R_1 = R_3 = 1M\Omega$ and $R_2 = 1k\Omega$. $F = 96,485C/mol$ is the Faraday constant.

$$O_2 + 2\,H_2O + 4\,e^- \rightleftharpoons 2\,H_2O_2 + 4\,e^- \rightleftharpoons 4\,(OH)^-$$

$$4\,(OH)^- + 4\,KCl \rightleftharpoons 4\,KOH + 4\,Cl^-$$

$$4\,Ag + 4\,Cl^- \rightleftharpoons 4\,AgCl + 4\,e^-$$

$$I = 4\,F\,[O_2]\,\phi$$



a) What output voltage Vout do you expect when the voltage source Vs is set to zero? Explain.

b) Now with the voltage source Vs set to $-0.7V$, find the sensitivity of the voltage output V_{out} to oxygen concentration $[O_2]$ in the sample at steady state.

c) Explain how the Ag/AgCl electrode gets consumed with the consumption of oxygen.

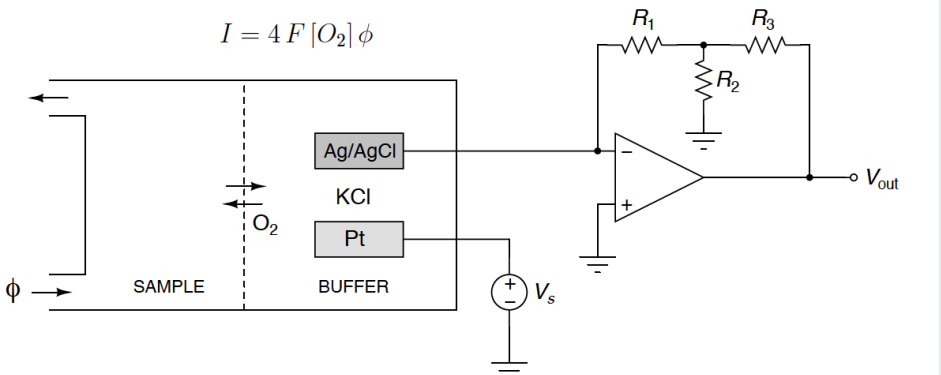
Self Study Question

$$O_2 + 2\,H_2O + 4\,e^- \rightleftharpoons 2\,H_2O_2 + 4\,e^- \rightleftharpoons 4\,(OH)^-$$

$$4\,(OH)^- + 4\,KCl \rightleftharpoons 4\,KOH + 4\,Cl^-$$

$$4\,Ag + 4\,Cl^- \rightleftharpoons 4\,AgCl + 4\,e^-$$

$$I = 4\,F\,[O_2]\,\phi$$



a) No electrons are injected by the platinum electrode into the solution so the current is $I = 0$.

b) $V_{out} = R_f I = \frac{R_1 R_3}{R_2} 4F[O_2]\phi$

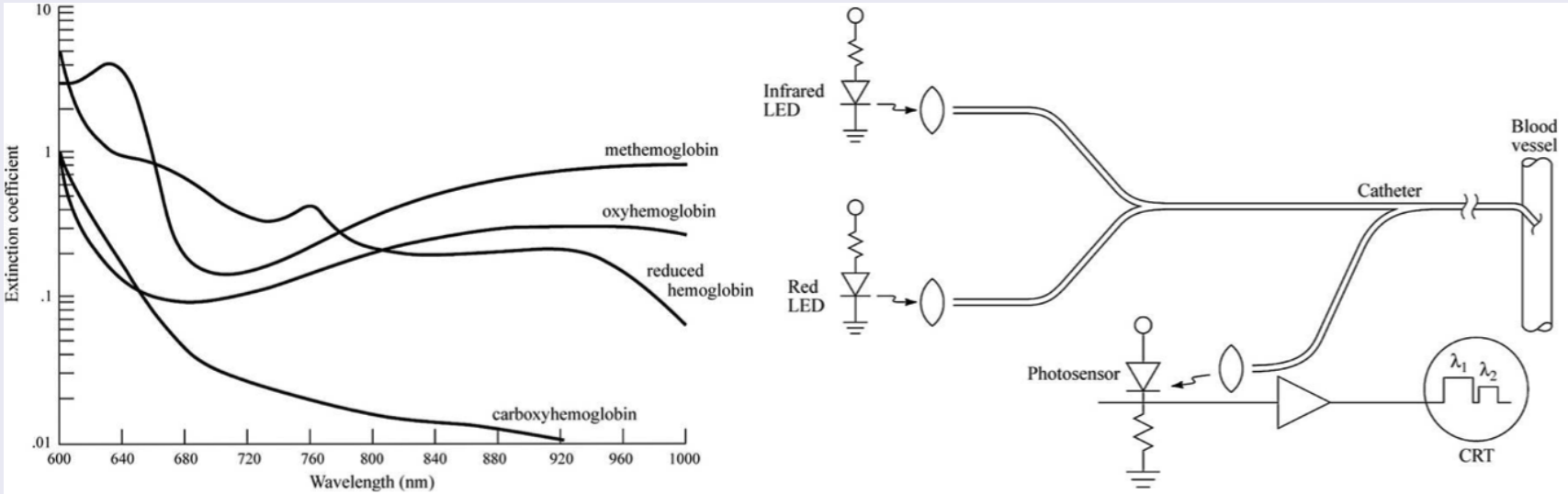
$$S = \frac{\partial V_{out}}{\partial [O_2]} = \frac{R_1 R_3}{R_2} 4F\phi = \frac{1 \cdot 10^6 \cdot 1 \cdot 10^6}{1 \cdot 10^3} 4 \cdot 96485 \cdot 10^{-3} = 4 \cdot 10^{11}\, V/(mol/l)$$

c) For every mole of O_2, 4 moles of Ag converts into $AgCl$.

Optical Biochemical Transducers

O_2 concentration can be monitored by an intravascular fiber optic catheter loacated in the pulmonary artery during cardiac surgery and in the ICU.

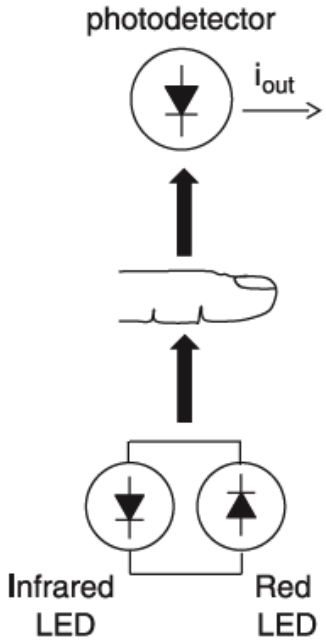
Optical absorbtion spectra indicates that 805 nm yields a measurement independent of the degree of oxygenation.

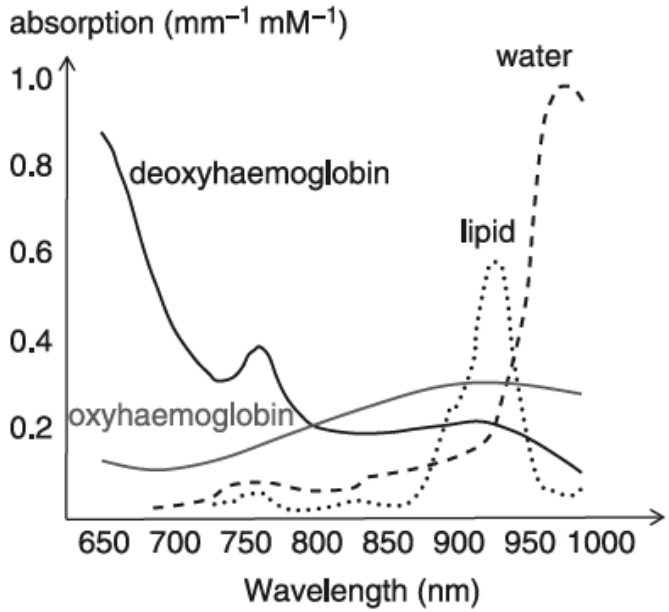


Oxygen saturation is masured by taking the ratio of diffuse backscattered light intensities at 660nm and 805 nm.

$A(\lambda) = WL[a_o(\lambda)C_o + a_r(\lambda)C_r]$
where W: weight of hemoglobin / unit volume,
L: optical path length,
(a_o, C_o), (a_r, C_r): absorptivities and concentrations of HbO_2 and Hb where $C_o + C_r = 1$ and $C_o = S_{O_2}$
Since $a_o = a_r = a$ at $\lambda_2 = 805$nm
$WL = \frac{A(\lambda_2)}{a(\lambda_2)}$ and $A(\lambda) = \frac{A(\lambda_2)}{a(\lambda_2)}[a_0(\lambda)C_0 + a_r(\lambda)C_r]$
When absorbance is measured at a second wavelength λ_1, the oxygen saturation is given by
$C_o = x + y\frac{A(\lambda_1)}{A(\lambda_2)}$
$x = \frac{a_r(\lambda_1)}{a_r(\lambda_1) - a_o(\lambda_1)}$ and $y = -\frac{a(\lambda_2)}{a_r(\lambda_1) - a_o(\lambda_1)}$
are constants reflecting the optical characteristics of blood.
λ_1 is usually chosen at wavelength where the difference between a_0 and a_r is maximum which occurs at 660nm.

Pulse Oximeter as a Noninvasive Blood Oxygen Monitoring





According to *Beer-Lambert Law*

$$I = I_0 e^{-\alpha C d}$$

where I and I_0 are the measured and incident light intensities, α is the absorbtion coefficient and d is the path length of the light through the tissue.

Measured intensity at 660nm I_R is

$$I_R = I_{0,R} e^{-\alpha_{R,tissue}[tissue]d - \alpha_{R,Hb}[Hb]d - \alpha_{R,HbO_2}[HbO_2]d}$$

Pulse Oximeter as a Noninvasive Blood Oxygen Monitoring

To eliminate the time varying amount of light through the tissue due to heartbeat

$$\frac{I_{R,max}}{I_{R,min}} = e^{\alpha_{R,HbO_2}[HbO_2](d_2-d_1)+\alpha_{R,Hb}[Hb](d_2-d_1)}$$

where d_1 and d_2 are different tissue dimensions through which the light travels during the heart cycle.

If the transmitted infrared light is I_{IR}

$$\frac{I_{IR,max}}{I_{IR,min}} = e^{\alpha_{IR,HbO_2}[HbO_2](d_2-d_1)+\alpha_{IR,Hb}[Hb](d_2-d_1)}$$

The red to infrared pulse modulation ratio R is

$$R = \frac{\ln \frac{I_{R,max}}{I_{R,min}}}{\ln \frac{I_{IR,max}}{I_{IR,min}}} = \frac{\alpha_{R,HbO_2}[HbO_2]+\alpha_{R,Hb}[Hb]}{\alpha_{IR,HbO_2}[HbO_2]+\alpha_{IR,Hb}[Hb]}$$

Remembering that $S_{O_2} = \frac{[HbO_2]}{[HbO_2]+[Hb]}$

$$S_{O_2} = \frac{\alpha_{R,Hb}-\alpha_{R,HbO_2}R}{\alpha_{R,Hb}-\alpha_{R,HbO_2}+(\alpha_{R,HbO_2}+\alpha_{IR,Hb})R}$$

For calibration the quadratic polynomial

$$S_{O_2} = a + bR + cR^2$$

is used for fitting in order to find a, b and c.

Self Study Question

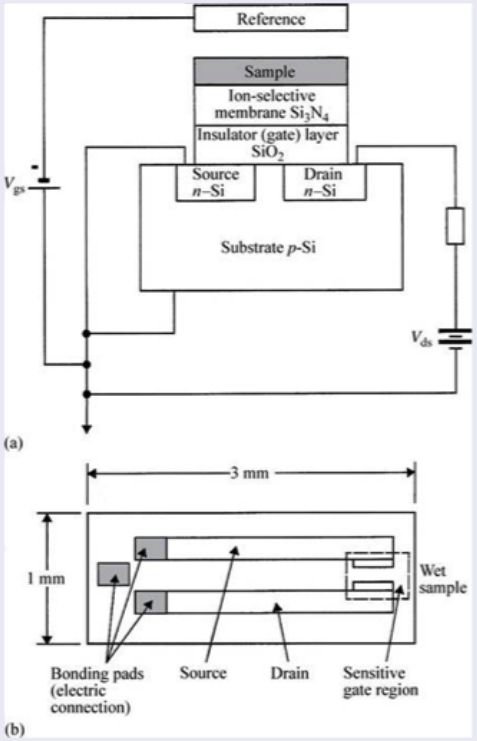
A pulse oximeter is used to measure oxygen saturation in the blood. Given spectral measurements of the absorptivities $a_O(\lambda)$ and $a_r(\lambda)$ of oxygenated and reduced hemoglobin as a function of wavelength λ, which coincide at the isosbestic wavelength λ_i (i.e., $a_i = a_o(\lambda_i) = a_r(\lambda_i)$).

Find an expression for S_{O2} as a function of measurements of absorbance in in the vessel at two wavelengths, $A(\lambda)$ and $A(\lambda_i)$ where $\lambda_i \neq \lambda$. How would you choose λ to maximize sensitivity.

$$A(\lambda) = W\ L\ a(\lambda) = W\ L\left(a_o(\lambda)S_{O_2} + a_r(\lambda)(1 - S_{O_2})\right)$$
$$A(\lambda_i) = W\ La_i$$
$$\frac{A(\lambda)}{A(\lambda_i)} = \frac{a_o(\lambda)S_{O_2} + a_r(\lambda)(1-S_{O_2})}{a_i} = \frac{(a_o(\lambda) - a_r(\lambda))}{a_i} S_{O_2} + \frac{a_r(\lambda)}{a_i}$$
$$S_{O_2} = \frac{a_i}{a_o(\lambda) - a_r(\lambda)} \frac{A(\lambda)}{A(\lambda_i)} - \frac{a_r(\lambda)}{a_o(\lambda) - a_r(\lambda)}$$

FET Integrated Biosensors

They are compact ($\ll 1\text{mm}^2$) electrochemical sensors where ion specific electrodes are formed on the gate of a FET and the concentration modulates the current or the conductance of its channel.
They can be integrated with standard transistor circuits on silicon microchips for automated high throughput screening for large number of biomarkers with high specificity.



In a chemically sensitive FET, the ion selective membrane modulates the current between the source and the drain.

A stretched ISFET maximizes the spacing between the wet sample region and the electric connections.

Simple model of channel conductance $g_{DS} = \frac{\partial I_D}{\partial V_{DS}}$

$$g_{DS} = \frac{W}{L} \underbrace{\mu}_{\text{electron mobility } (\mathrm{m}^2/\mathrm{Vs})} \overbrace{Q_{ox}}^{\text{surface charge density } (\mathrm{C}/\mathrm{m}^2)}$$

where W and L are the dimensions of the gate.

$$I_D = WQ_{ox}v \text{ and } v = \mu E \approx \mu \frac{V_{DS}}{L} \text{ for small } V_{DS}$$

$$Q_{ox} = C_{ox}(V_{GS} - V_{Th})$$

where $V_{Th} \approx 0.7V$ and $C_{ox} \approx 3.5 \cdot 10^{-11}/d$ in which d is the distance between gate and Si channel.

$$g_{DS} = \frac{W}{L}\mu C_{ox}(V_{GS} - V_{Th})$$

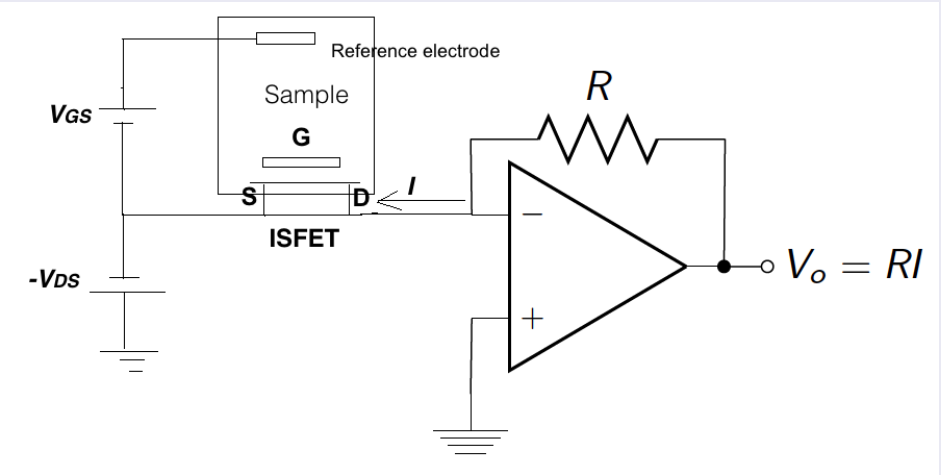
ISFET

Ion sensitive FET is effectively the same as a MOSFET where the gate is replaced with an *ion sensitive membrane*.
As an example, silicon nitride (Si_3N_4) is sensitive to pH selectively passing H^+.
The net ion charge diffusing through the membrane is approximately proportional to the ion concentration in the sample. Other membranes are selective to other ion types.

$$Q_{ox} \approx k[H^+] \longrightarrow g_{DS} \approx \frac{W}{L}\mu k[H^+]$$

which can be measured by a Wheatstone bridge or Potentiostat at a constant small voltage bias ($V_{DS} \ll V_{Th}$)

ISFET with Potentiostat Biasing



$$V_0 = RI \approx Rg_{DS}V_{DS}$$
$$g_{DS} \approx \frac{W}{L}\mu k[H^+]$$

Immunologically Sensitive FET (IMFET)

A special ISFET where the membrane is impermeable to ions but is coated with antibodies for specific antigen detection or coated with antigens for specific antibody detection.

$g_{DS} \approx \frac{W}{L}\mu kN[A^+]$ where N : the number of binding sites per antibody

A^+ : antigen concentration

Arrays of various antibody labeled IMFETS integrated with MOSFET amplification and selection circuits allow for high throughput pathogen detection on a single chip