Reading 4:
Chemical Biosensors: Electrical and Optical

In order to measure and record potentials and, hence, currents in the body, it is necessary to provide some interface between the body and the electronic measuring apparatus. Biopotential electrodes carry out this interface function. In any practical measurement of potentials, current flows in the measuring circuit for at least a fraction of the period of time over which the measurement is made. Ideally this current should be very small. However, in practical situations, it is never zero. Biopotential electrodes must therefore have the capability of conducting a current across the interface between the body and the electronic measuring circuit.

Our first impression is that this is a rather simple function to achieve and that biopotential electrodes should be relatively straightforward. But when we consider the problem in more detail, we see that the electrode actually carries out a transducing function, because in the body current is carried by ions, whereas in the electrode and its lead wire it is carried by electrons. Thus the electrode must serve as a transducer to change an ionic current into an electronic current. This greatly complicates electrodes and places constraints on their operation. We shall briefly examine the basic mechanisms involved in the transduction process and shall look at how they affect electrode characteristics. We shall next examine the principal electrical characteristics of biopotential electrodes and discuss electrical equivalent circuits for electrodes based on these characteristics. We shall then cover some of the different forms that biopotential electrodes take in various types of medical instrumentation systems. Finally, we shall look at electrodes used for measuring the ECG, EEG, EMG, and intracellular potentials.

5.1 THE ELECTRODE–ELECTROLYTE INTERFACE

The passage of electric current from the body to an electrode can be understood by examining the electrode–electrolyte interface that is schematically illustrated in Figure 5.1. The electrolyte represents the body fluid containing ions. A net current that crosses the interface, passing from the electrode to the electrolyte, consists of (1) electrons moving in a direction opposite to that of the current in the electrode, (2) cations (denoted by C^+) moving in the same direction as the current, and (3) anions (denoted by A^-) moving in a direction opposite to that of the current in the electrolyte.

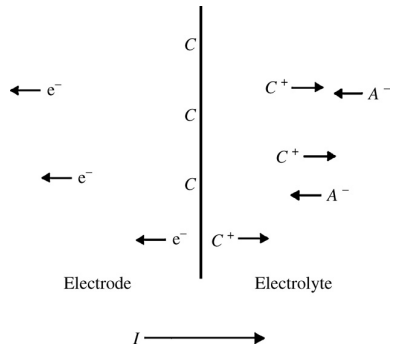


Figure 5.1 Electrode–electrolyte interface The current crosses it from left to right. The electrode consists of metallic atoms C. The electrolyte is an aqueous solution containing cations of the electrode metal C^+ and anions A^-.

For charge to cross the interface—there are no free electrons in the electrolyte and no free cations or anions in the electrode—something must occur at the interface that transfers the charge between these carriers. What actually occur are chemical reactions at the interface, which can be represented in general by the following reactions:

$$C \rightleftarrows C^{n+} + ne^- \tag{5.1}$$

$$A^{m-} \rightleftarrows A + me^- \tag{5.2}$$

where n is the valence of C and m is the valence of A. Note that in (5.1) we are assuming that the electrode is made up of some atoms of the same material as the cations and that this material in the electrode at the interface can become oxidized to form a cation and one or more free electrons. The cation is discharged into the electrolyte; the electron remains as a charge carrier in the electrode.

The reaction involving the anions is given in (5.2). In this case an anion coming to the electrode–electrolyte interface can be oxidized to a neutral atom, giving off one or more free electrons to the electrode.

Note that both reactions are often reversible and that reduction reactions (going from right to left in the equations) can occur as well. As a matter of fact, when no current is crossing the electrode–electrolyte interface, these reactions often still occur. But the rate of oxidation reactions equals the rate of reduction reactions, so the net transfer of charge across the interface is zero. When the current flow is from electrode to electrolyte, as indicated in Figure 5.1, the oxidation reactions dominate. When the current is in the opposite direction, the reduction reactions dominate.

To further explore the characteristics of the electrode–electrolyte interface, let us consider what happens when we place a piece of metal into a solution containing ions of that metal. These ions are cations, and the solution, if it is to maintain neutrality of charge, must have an equal number of anions. When the metal comes in contact with the solution, the reaction represented by (5.1) begins immediately. Initially, the reaction goes predominantly either to the left or to the right, depending on the concentration of cations in solution and the equilibrium conditions for that particular reaction. The local concentration of cations in the solution at the interface changes, which affects the anion concentration at this point as well. The net result is that neutrality of charge is not maintained in this region. Thus the electrolyte surrounding the metal is at a different electric potential from the rest of the solution. A potential difference known as the *half-cell potential* is determined by the metal involved, the concentration of its ions in solution, and the temperature, as well as other second-order factors. Knowledge of the half-cell potential is important for understanding the behavior of biopotential electrodes.

The distribution of ions in the electrolyte in the immediate vicinity of the metal–electrolyte interface has been of great interest to electrochemists, and several theories have been developed to describe it. Geddes (1972) compares the charges and potential distributions for four of these theories, whereas Cobbold (1974), in a discussion of the half-cell potential, considers the Stern model. Rather than analyze these theories here, we shall accept their general conclusion. Some separation of charges at the metal–electrolyte interface results in an electric double layer, wherein one type of charge is dominant on the surface of the metal, and the opposite charge is distributed in excess in the immediately adjacent electrolyte. This charge distribution at the electrode–electrolyte interface can affect electrode performance, as we will see in Section 5.3.

It is not possible to measure the half-cell potential of an electrode because—unless we use a second electrode—we cannot provide a connection between the electrolyte and one terminal of the potential-measuring apparatus. Because this second electrode also has a half-cell potential, we merely end up measuring the difference between the half-cell potential of the metal and that of the second electrode. There would of course be a very large number of combinations of pairs of electrodes, so tabulations of such differential half-cell potentials would be very extensive. To avoid this problem, electrochemists have adopted the standard convention that a particular electrode—the hydrogen electrode—is defined as having a half-cell potential of zero under conditions that are achievable in the laboratory. We can then measure the half-cell potentials of all other electrode materials with respect to this electrode.

Table 5.1 lists several common materials that are used for electrodes and gives their half-cell potentials. Table 5.1 also gives the oxidation–reduction reactions that occur at the surfaces of these electrodes and enable us to arrive at the potentials. The hydrogen electrode is based on the reaction

$$H_2 \rightleftarrows 2H \rightleftarrows 2H^+ + 2e^- \tag{5.3}$$

Table 5.1 Half-cell Potentials for Common Electrode Materials at 25 °C

The metal undergoing the reaction shown has the sign and potential E^0 when referenced to the hydrogen electrode

Metal and Reaction	Potential E^0 (V)
$Al \rightarrow Al^{3+} + 3e^-$	−1.706
$Zn \rightarrow Zn^{2+} + 2e^-$	−0.763
$Cr \rightarrow Cr^{3+} + 3e^-$	−0.744
$Fe \rightarrow Fe^{2+} + 2e^-$	−0.409
$Cd \rightarrow Cd^{2+} + 2e^-$	−0.401
$Ni \rightarrow Ni^{2+} + 2e^-$	−0.230
$Pb \rightarrow Pb^{2+} + 2e^-$	−0.126
$H_2 \rightarrow 2H^+ + 2e^-$	0.000 by definition
$Ag + Cl^- \rightarrow AgCl + e^-$	+0.223
$2Hg + 2Cl^- \rightarrow Hg_2Cl_2 + 2e^-$	+0.268
$Cu \rightarrow Cu^{2+} + 2e^-$	+0.340
$Cu \rightarrow Cu^+ + e^-$	+0.522
$Ag \rightarrow Ag^+ + e^-$	+0.799
$Au \rightarrow Au^{3+} + 3e^-$	+1.420
$Au \rightarrow Au^+ + e^-$	+1.680

SOURCE: Data from *Handbook of Chemistry and Physics,* 55th ed., Cleveland, OH: CRC Press, 1974–1975, with permission.

where H_2 gas bubbled over a platinum electrode is the source of hydrogen molecules. The platinum also serves as a catalyst for the reaction on the left-hand side of the equation and as an acceptor of the generated electrons.

5.2 POLARIZATION

The half-cell potential of an electrode is described in Section 5.1 for conditions in which no electric current exists between the electrode and the electrolyte. If, on the other hand, there is a current, the observed half-cell potential is often altered. The difference is due to polarization of the electrode. The difference between the observed half-cell potential and the equilibrium zero-current half-cell potential is known as the *overpotential.* Three basic mechanisms contribute to this phenomenon, and the overpotential can be separated into three components: the ohmic, the concentration, and the activation overpotentials.

The *ohmic overpotential* is a direct result of the resistance of the electrolyte. When a current passes between two electrodes immersed in an electrolyte, there is a voltage drop along the path of the current in the electrolyte as a result of its resistance. This drop in voltage is proportional to the current and the resistivity of the electrolyte. The resistance between the electrodes can itself vary as a function of the current. Thus the ohmic overpotential does not

necessarily have to be linearly related to the current. This is especially true in electrolytes having low concentrations of ions. This situation, then, does not necessarily follow Ohm's law.

The *concentration overpotential* results from changes in the distribution of ions in the electrolyte in the vicinity of the electrode–electrolyte interface. Recall that the equilibrium half-cell potential results from the distribution of ionic concentration in the vicinity of the electrode–electrolyte interface when no current flows between the electrode and the electrolyte. Under these conditions, reactions (5.1) and (5.2) reach equilibrium, so the rates of oxidation and reduction at the interface are equal. When a current is established, this equality no longer exists. Thus it is reasonable to expect the concentration of ions to change. This change results in a different half-cell potential at the electrode. The difference between this and the equilibrium half-cell potential is the concentration overpotential.

The third mechanism of polarization results in the *activation overpotential.* The charge-transfer processes involved in the oxidation–reduction reaction (5.1) are not entirely reversible. In order for metal atoms to be oxidized to metal ions that are capable of going into solution, the atoms must overcome an energy barrier. This barrier, or *activation energy,* governs the kinetics of the reaction. The reverse reaction—in which a cation is reduced, thereby plating out an atom of the metal on the electrode—also involves an activation energy, but it does not necessarily have to be the same as that required for the oxidation reaction. When there is a current between the electrode and the electrolyte, either oxidation or reduction predominates, and hence the height of the energy barrier depends on the direction of the current. This difference in energy appears as a difference in voltage between the electrode and the electrolyte, which is known as the *activation overpotential.*

These three mechanisms of polarization are additive. Thus the net overpotential of an electrode is given by

$$V_p = E^0 + V_r + V_c + V_a \tag{5.4}$$

where

V_p = total potential, or polarization potential, of the electrode
E^0 = half-cell potential
V_r = ohmic overpotential
V_c = concentration overpotential
V_a = activation overpotential

When an ion-selective semipermeable membrane separates two aqueous ionic solutions of different concentration, an electric potential exists across this membrane. It can be shown (Plonsey and Barr, 2007) that this potential is given by the Nernst equation

$$E = -\frac{RT}{nF}\ln\frac{a_1}{a_2} \tag{5.5}$$

where a_1 and a_2 are the activities of the ions on each side of the membrane. [Other terms are defined in (4.1) and the Appendix.] In dilute solutions, ionic activity is approximately equal to ionic concentration. When intermolecular effects become significant, which happens at higher concentrations, the activity of the ions is less than their concentration.

The half-cell potentials listed in Table 5.1 are known as the standard half-cell potentials because they apply to standard conditions. When the electrode–electrolyte system no longer maintains this standard condition, half-cell potentials different from the standard half-cell potential are observed. The differences in potential are determined primarily by temperature and ionic activity in the electrolyte. *Ionic activity* can be defined as the availability of an ionic species in solution to enter into a reaction.

The standard half-cell potential is determined at a standard temperature; the electrode is placed in an electrolyte containing cations of the electrode material having unity activity. As the activity changes from unity (as a result of changing concentration), the half-cell potential varies according to the Nernst equation:

$$E = E^0 + \frac{RT}{nF}\ln(a_{c^{n+}}) \tag{5.6}$$

where

E = half-cell potential
E^0 = standard half-cell potential
n = valence of electrode material
$a_{c^{n+}}$ = activity of cation C^{n+}

Equation (5.6) represents a specific application of the Nernst equation to the reaction of (5.1). The more general form of this equation can be written for a general oxidation–reduction reaction as

$$\alpha A + \beta B \rightleftarrows \gamma C + \delta D + n\mathrm{e}^- \tag{5.7}$$

where n electrons are transferred. The general Nernst equation for this situation is

$$E = E^0 = \frac{RT}{nF}\ln\frac{a_C^{\gamma}a_D^{\delta}}{a_A^{\alpha}a_B^{\beta}} \tag{5.8}$$

where the a's represent the activities of the various constituents of the reaction.

An electrode–electrolyte interface is not required for a potential difference to exist. If two electrolytic solutions are in contact and have different concentrations of ions with different ionic mobilities, a potential difference,

known as a *liquid-junction potential,* exists between them. For solutions of the same composition but different activities, its magnitude is given by

$$E_j = \frac{\mu_+ - \mu_-}{\mu_+ + \mu_-} \frac{RT}{nF} \ln \frac{a'}{a''} \tag{5.9}$$

where μ_+ and μ_- are the mobilities of the positive and negative ions, and a' and a'' are the activities of the two solutions. Though liquid-junction potentials are generally not so high as electrode–electrolyte potentials, they can easily be of the order of tens of millivolts. For example, two solutions of sodium chloride, at 25 °C, with activities that vary by a factor of 10, have a potential difference of approximately 12 mV. Note that you can generate potentials of the order of some biological potentials by merely creating differences in concentration in an electrolyte. This is a factor to consider when you are examining actual electrode systems used for biopotential measurements.

EXAMPLE 5.1 An electrode consisting of a piece of Zn with an attached wire and another electrode consisting of a piece of Ag coated with a layer of AgCl and an attached wire are placed in a 1 M $ZnCl_2$ solution (activities of Zn^{2+} and Cl^- are approximately unity) to form an electrochemical cell that is maintained at a temperature of 25 °C.

a. What chemical reactions might you expect to see at these electrodes?
b. If a very high input impedance voltmeter were connected between these electrodes, what would it read?
c. If the lead wires from the electrodes were shorted together, would a current flow? How would this affect the reactions at the electrodes?
d. How would you expect the voltage between the electrodes to differ from the equilibrium open-circuit voltage of the cell immediately following removal of the short circuit?

ANSWER

a. Zinc is much more chemically active than Ag, so the atoms on its surface oxidize to Zn^{2+} ions according to the reaction: $Zn \rightleftarrows Zn^{2+} + 2e^-$, which according to Table 5.1 has an E^0 of –0.763 V.

At the Ag electrode, Ag can be oxidized to form Ag^+ ions according to the reaction: $Ag \rightleftarrows Ag^+ + 1e^-$. These ions immediately react with the Cl^- ions in solution to form AgCl, $Ag^+ + Cl^- \rightleftarrows AgCl\downarrow$. Most of this precipitates out of solution due to this salt's low solubility. This reaction has an E^0 of 0.223 V at 25 °C.

b. When no current is drawn from or supplied to either electrode, and the concentration of ions is uniform throughout the solution, the difference in voltage between the electrodes is the difference between the half-cell potentials:

$$V = E^0_{Zn} - E^0_{Ag} = -0.763\,V - 0.233\,V = -0.986\,V$$

Because Zn oxidizes at a higher potential, the electrons remaining in it are at a higher energy than those in the Ag. Thus the Zn electrode has a negative voltage with respect to the Ag electrode.

c. There is a potential difference between the two electrodes, so there will be a current when they are shorted together. The flow of electrons is from the Zn to the Ag, because the Zn electrons are at a higher energy. Thus Zn is consumed and yields electrons, and AgCl absorbs electrons and plates out metallic Ag.

d. When the electrodes are connected, they must be at the same potential at the point of connection. Thus the 0.986 V half-cell potential difference must be opposed by polarization overpotentials and ohmic losses in the electrodes and connecting wires. When the connection is broken and the current stops, the ohmic overpotential and electrode losses become zero, but the concentration overpotential remains until the gradient of the ionic concentration at the electrode surfaces returns to its equilibrium value for zero current. Thus the difference in voltage between the two electrodes is less than 0.986 V when the circuit is opened but rises to that value asymptotically with time.

5.3 POLARIZABLE AND NONPOLARIZABLE ELECTRODES

Theoretically, two types of electrodes are possible: those that are perfectly polarizable and those that are perfectly nonpolarizable. This classification refers to what happens to an electrode when a current passes between it and the electrolyte. *Perfectly polarizable electrodes* are those in which no actual charge crosses the electrode–electrolyte interface when a current is applied. Of course, there has to be current across the interface, but this current is a displacement current, and the electrode behaves as though it were a capacitor. *Perfectly nonpolarizable electrodes* are those in which current passes freely across the electrode–electrolyte interface, requiring no energy to make the transition. Thus, for perfectly nonpolarizable electrodes there are no overpotentials.

Neither of these two types of electrodes can be fabricated; however, some practical electrodes can come close to acquiring their characteristics. Electrodes made of noble metals such as platinum come closest to behaving as perfectly polarizable electrodes. Because the materials of these electrodes are relatively inert, it is difficult for them to oxidize and dissolve. Thus current passing between the electrode and the electrolyte changes the concentration primarily of ions at the interface, so a majority of the overpotential seen from this type of electrode is a result of V_c, the concentration overpotential. The electrical characteristics of such an electrode show a strong capacitive effect.

THE SILVER/SILVER CHLORIDE ELECTRODE

The silver/silver chloride (Ag/AgCl) electrode is a practical electrode that approaches the characteristics of a perfectly nonpolarizable electrode and can

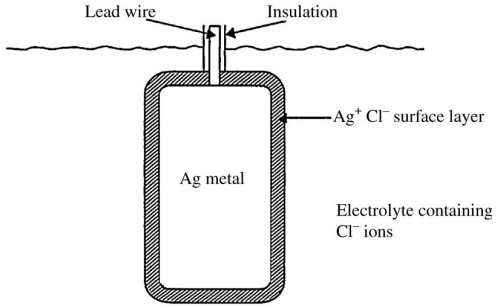


Figure 5.2 A silver/silver chloride electrode, shown in cross section.

be easily fabricated in the laboratory. It is a member of a class of electrodes each of which consists of a metal coated with a layer of a slightly soluble ionic compound of that metal with a suitable anion. The whole structure is immersed in an electrolyte containing the anion in relatively high concentrations.

The structure is shown in Figure 5.2. A silver metal base with attached insulated lead wire is coated with a layer of the ionic compound AgCl. (This material—AgCl—is only very slightly soluble in water, so it remains stable.) The electrode is then immersed in an electrolyte bath in which the principal anion of the electrolyte is Cl^-. For best results, the electrolyte solution should also be saturated with AgCl so that there is little chance for any of the surface film on the electrode to dissolve.

The behavior of the Ag/AgCl electrode is governed by two chemical reactions. The first involves the oxidation of silver atoms on the electrode surface to silver ions in solution at the interface.

$$Ag \rightleftarrows Ag^+ + e^- \tag{5.10}$$

$$Ag^+ + Cl^- \rightleftarrows AgCl\downarrow \tag{5.11}$$

The second reaction occurs immediately after the formation of Ag^+ ions. These ions combine with Cl^- ions already in solution to form the ionic compound AgCl. As mentioned before, AgCl is only very slightly soluble in water, so most of it precipitates out of solution onto the silver electrode and contributes to the silver chloride deposit. Silver chloride's rate of precipitation and of returning to solution is a constant K_s known as the *solubility product.*

Under equilibrium conditions the ionic activities of the Ag^+ and Cl^- ions must be such that their product is the solubility product.

$$a_{Ag^+} \times a_{Cl^-} = K_s \tag{5.12}$$

In biological fluids the concentration of Cl^- ions is relatively high, which gives it an activity just a little less than unity. The solubility product for AgCl, on the other hand, is of the order of 10^{-10}. This means that, when an Ag/AgCl electrode is in contact with biological fluids, the activity of the Ag^+ ion must be very low and of the same order of magnitude as the solubility product.

We can determine the half-cell potential for the Ag/AgCl electrode by writing (5.6) for the reaction of (5.10).

$$E = E^0_{Ag} + \frac{RT}{nF} \ln a_{Ag^+} \tag{5.13}$$

By using (5.12), we can rewrite this as

$$E = E^0_{Ag} + \frac{RT}{nF} \ln \frac{K_s}{a_{Cl^-}} \tag{5.14}$$

or

$$E = E^0_{Ag} + \frac{RT}{nF} \ln K_s - \frac{RT}{nF} \ln a_{Cl^-} \tag{5.15}$$

The first and second terms on the right-hand side of (5.15) are constants; only the third is determined by ionic activity. In this case, it is the activity of the Cl^- ion, which is relatively large and not related to the oxidation of Ag, which is caused by the current through the electrode. The half-cell potential of this electrode is consequently quite stable when it is placed in an electrolyte containing Cl^- as the principal anion, provided the activity of the Cl^- remains stable. Because this is the case in the body, we shall see in later sections of this chapter that the Ag/AgCl electrode is relatively stable in biological applications.

There are several procedures that can be used to fabricate Ag/AgCl electrodes (Janz and Ives, 1968). Two of them are of particular importance in biomedical electrodes. One is the electrolytic process for forming Ag/AgCl electrodes. An electrochemical cell is made up in which the Ag electrode on which the AgCl layer is to be deposited serves as anode and another piece of Ag—having a surface area much greater than that of the anode—serves as cathode. A 1.5 V battery serves as the energy source, and a series resistance limits the peak current, thereby controlling the maximal rate of reaction. A milliammeter can be placed in the circuit to observe the current, which is proportional to the rate of reaction.

The reactions of (5.10) and (5.11) begin to occur as soon as the battery is connected, and the current jumps to its maximal value. As the thickness of the deposited AgCl layer increases, the rate of reaction decreases and the current drops. This situation continues, and the current approaches zero asymptotically. Theoretically, the reaction is not complete until the current drops to zero. In practice this never occurs because of other processes going on that conduct a current. Therefore the reaction can be stopped after a few minutes, once the current has reached a relatively stable low value—of the order of 10 μA for most biological electrodes.

EXAMPLE 5.2 An AgCl surface is grown on an Ag electrode by the electrolytic process described in the previous paragraph. The current passing through the cell is measured and recorded during the growth of the AgCl layer and is found to be represented by the equation

$$I = 100\,\text{mA}\,e^{-t/10\,\text{s}} \tag{E5.1}$$

a. If the reaction is allowed to run for a long period of time, so that the current at the end of this period is essentially zero; how much charge is removed from the battery during this reaction?
b. How many grams of AgCl are deposited on the Ag electrode's surface by this reaction?
c. The chloride electrode is now placed into a beaker containing 1 liter of 0.9 molar NaCl solution. How much AgCl will be dissolved?

ANSWER

a. The total charge crossing the electrode–electrolyte interface during the reaction is

$$q = \int_0^\infty i\,dt = 100\,\text{mA}\int_0^\infty e^{-t/10}dt = 1\,\text{C} \tag{E5.2}$$

b. One molecule of AgCl is deposited for each electron. The number of atoms deposited is

$$N = \frac{1\,\text{C}}{1.6\times10^{-19}\text{C/atom}} = 6.25\times10^{8}\,\text{atoms} \tag{E5.3}$$

The number of moles can be found by dividing by Avogadro's number.

$$N = \frac{6.25\times10^{18}}{6.03\times10^{23}} = 1.036\times10^{-5}\,\text{mol} \tag{E5.4}$$

The molecular weight of AgCl is 143.2, therefore the mass of AgCl formed is

$$142.3\times1.036\times10^{-5} = 1.47\times10^{-3}\,\text{g} \tag{E5.5}$$

c. For AgCl the solubility product is $K_s = 1.56\times10^{-10}$ at 25 °C. The activity and concentration are about the same at these low concentrations. Thus

$$[\text{Ag}^+][\text{Cl}^-] = 1.56\times10^{-10} \tag{E5.6}$$

Since $[\text{Cl}^-]$ in the NaCl solution is 0.9 mole/liter, the dissolved Ag will be

$$[\text{Ag}^+] = 1.73\times10^{-10}\,\text{mol/liter}$$

In terms of mass this will be $1.73\times10^{-10}\times142.3 = 2.46\times10^{-8}$ g.

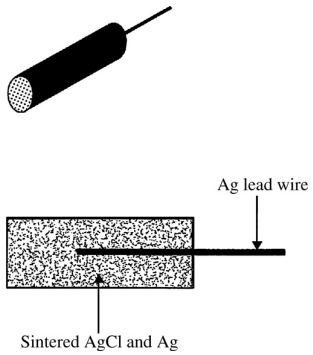


Figure 5.3 Sintered Ag/AgCl electrode

The second process for producing Ag/AgCl electrodes useful in medical instrumentation is a sintering process that forms pellet electrodes, as shown in Figure 5.3. The electrode consists of an Ag lead wire surrounded by a sintered Ag/AgCl cylinder. It is formed by placing the cleaned lead wire in a die that is then filled with a mixture of powdered Ag and AgCl. The die is compressed in an arbor press to form the powdered components into a pellet, which is then removed from the die and baked at 400 °C for several hours. These electrodes tend to have a greater endurance than the electrolytically deposited AgCl electrodes, and they are best applied when repeated usage is necessary. The electrolytically deposited AgCl has a tendency to flake off under mechanical stress, leaving portions of metallic Ag in contact with the electrolyte, which can cause the electrode's half-cell, potential to be unstable and noisy.

Silver chloride is not a very good conductor of an electric current. If the powder that was compressed to make the sintered electrode consisted only of finely ground silver chloride, this would result in a high-resistance electric connection between the electrolytic solution and the silver wire in the center of the sintered electrode. Electrochemists found that they could increase the conductivity of the silver chloride pellet by including metallic silver powder along with the silver chloride powder. The amount of metallic silver is small enough to make highly unlikely any direct connection from the silver wire to the electrode through silver particles. Instead, there is always some silver chloride between the silver particles, but the presence of the silver particles makes it easier for current to pass through the silver chloride.

A similar situation occurs in the electrolytically prepared silver/silver chloride electrode. Although the silver chloride layer is much thinner in this case than it is for the sintered electrode, it remains a pure silver chloride layer for only a short time after it is deposited. Silver chloride is a silver-halide salt, and these materials are photosensitive. Light striking these salts can cause the silver ions to be reduced to metallic silver atoms. Thus, for all practical purposes, the electrolytically deposited silver chloride layer contains fine silver particles as well. Evidence of their presence appears when the layer is grown

and immediately becomes dark gray because of the fine silver particles (pure silver chloride is amber colored).

In addition to its nonpolarizable behavior, the Ag/AgCl electrode exhibits less electric noise than the equivalent metallic Ag electrodes. Geddes and Baker (1989) showed that electrodes with the AgCl layer exhibited far less noise than was observed when the AgCl layer was removed. Also, a majority of the noise for the purely metallic electrodes was at low frequencies. This would provide the most serious interference for low-frequency, low-voltage recordings, such as the EEG.

A second kind of electrode that has characteristics approaching those of the perfectly nonpolarizable electrode is the *calomel electrode.* It is used primarily as a reference electrode for electrochemical determinations and is frequently applied as the reference electrode when pH is measured (see Section 10.2). The calomel electrode is often constructed as a glass tube with a porous glass plug at its base filled with a paste of mercurous chloride or calomel (Hg_2Cl_2) mixed with a saturated potassium chloride (KCl) solution. Like AgCl, the Hg_2Cl_2 is only slightly soluble in water, so most of it retains its solid form. A layer of elemental mercury is placed on top of the paste layer with an electric lead wire within it. This entire assembly is then positioned in the center of a larger glass tube with a porous glass plug at its base. The tube is filled with a saturated KCl solution so that the Hg_2Cl_2 layer of the inner tube is in contact with this electrolyte through the porous plug of the inner tube. We have a half-cell made up of Hg in intimate contact with an Hg_2Cl_2 layer that is in contact with the saturated KCl electrolyte. The porous plug at the bottom of the electrode assembly is used to make contact between the internal KCl solution and the solution in which the electrode is immersed. This is actually a liquid–liquid junction that can result in a liquid–liquid junction potential, which will add to the electrode half-cell potential.

Silver/silver chloride electrodes can be fabricated in the same form as the calomel electrode and used for electroanalytical chemical measurements. In this case, the mercury is replaced by silver and AgCl replaces the Hg_2Cl_2 in the electrode structure.

Using the same argument as that used for the Ag/AgCl electrode, we can show that the half-cell potential of this electrode is dependent on the Cl^- activity in the saturated KCl solution. This is stable at a given temperature, because the solution is saturated and therefore has a stable chloride ion activity. In application, the tip of this electrode assembly that contains the porous plug is dipped into the electrolytic solution that it is to contact. In pH measurements, a pH electrode is also dipped into the solution, and the potential difference between the two electrodes is measured.

Chemical Biosensors

A chemical biosensor is a sensor that produces an electric signal proportional to the concentration of biochemical analytes. These biosensors use chemical as well as physical principles in their operation.

The body is composed of living cells. These cells, which are essentially chemical factories, the input to which is metabolic food and the output waste products, are the building blocks for the organ systems in the body. The functional status of an organ system is determined by measuring the chemical input and output analytes of the cells. As a consequence, the majority of tests made in the hospital or the physician's office deal with analyzing the chemistry of the body.

The important critical-care analytes are the blood levels of pH; P_{O_2}; P_{CO_2}; hematocrit; total hemoglobin; O_2 saturation; electrolytes including sodium, potassium, calcium, and chloride; and various metabolites including glucose, lactate, creatinine, and urea. Table 10.1 gives the normal ranges in blood for these critical-care analytes.

These variables are normally analyzed in a central clinical-chemistry laboratory remote from the patient's bedside. This conventional approach provides only historical values of the patient's blood chemistry, because there is a delay between when the sample is obtained and when the result is reported. (The sample must be transported to the main clinical-chemistry laboratory, and the appropriate analyses must be performed.) This inherent delay is approximately 30 min or more. Other significant drawbacks plague central-laboratory analyses of patient chemistry, including potential errors in the origin of the sample and in sample-handling techniques, and (because of the delay) the timeliness of the therapeutic intervention.

For these reasons, there has been a movement to decentralize clinical testing of the patient's chemistry (Collison and Meyerhoff, 1990). This is particularly important in the critical-care and surgical settings. The decentralized approach has resulted from a number of improvements in biosensor technology, including the development of blood-gas and electrolyte monitoring systems equipped with self-calibration for measuring the patient's blood chemistry at the bedside.

Economic pressures have also encouraged movement of sophisticated chemical-analysis and diagnostic equipment from the central laboratory to

Table 10.1 Critical-Care Analytes and Their Normal Ranges in Blood

Blood Gases and Related Parameters		Electrolytes		Metabolites	
P_{O_2}	80–104 mm Hg	Na^+	135–155 mmol/l	Glucose	70–110 mg/100 ml
P_{CO_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%				

SOURCE: M. E. Collison and M. E. Meyerhoff, ''Chemical sensors for bedside monitoring of critically ill patients,'' *Anal. Chem.*, 1990, 62, 425A–437A.

specific clinical areas. Such sites include the operating room, where patient blood gases and electrolytes must be monitored continuously, and dialysis centers, where patients are treated on an outpatient basis and measurements of uric acid and other blood analytes must be made in a timely manner. In addition, self-contained, small, economical blood-chemistry units have been developed for use in the physician's office and the patient's home.

In the future, integrated-circuit and optoelectronic technology will be used to develop miniaturized biosensors, which are sensitive to body analytes for real-time, *in vivo* measurements of body chemistry (Turner *et al.*, 1987). Self-contained biosensor units for closed-loop drug-delivery systems will also become available. Examples of future applications of closed-loop systems with chemical biosensors include (1) control of implantable pacemakers and defibrillators, (2) regulation of anesthesia during operations, and (3) control of insulin secretion from an artificial pancreas. Note that moving laboratory devices from a central location to a decentralized location in the hospital, physician's office, or patient's home poses significant challenges. These involve stability, calibration, quality control of the measurements, and ease of instrument use.

Noninvasive measurement of the biochemistry of the body will increase tremendously in the future. The advances in and burgeoning applications of pulse oximetry offer just one example of the impact that noninvasive measurement can have on patient monitoring. Pulse oximetry has become the standard of care in a number of clinical situations, which include monitoring during administration of anesthesia (to assess functioning of the cardiopulmonary system) and during the administration of oxygen to neonates (to avoid high arterial oxygen levels, which can lead to serious damage to retinal and pulmonary tissue). The future will see applications for noninvasive monitoring of the blood biochemistry in the standard blood-chemistry tests for glucose, cholesterol, urea, electrolytes, and so on.

10.1 BLOOD-GAS AND ACID–BASE PHYSIOLOGY

The fast and accurate measurements of the blood levels of the partial pressure of oxygen (P_{O_2}), the partial pressure of CO_2 (P_{CO_2}), and the concentration of hydrogen ions (pH) are vital in the diagnosis and treatment of many pathological conditions. Significant abnormalities of these quantities can rapidly be fatal if not treated appropriately. These measurements are usually made on specimens of arterial blood, though ''arterialized'' venous samples are often obtained from infants.

Oxygen is carried in the blood in two separate states. Normally, approximately 98% of the O_2 in the blood is combined with hemoglobin (Hb) in the red blood cells. The remaining 2% is physically dissolved in the plasma. The amount (saturation, S) of O_2 bound to Hb in arterial blood is defined as the ratio of the concentration of oxyhemoglobin (HbO_2) to the total concentration of Hb. That is,

$$S_{O_2}(\%) = \frac{[HbO_2]}{[\text{total Hb}]} \times 100 \tag{10.1}$$

The sigmoid-shaped oxyhemoglobin dissociation curve (ODC), shown in Figure 10.1, graphically illustrates the relationship between the percent oxygen saturation of hemoglobin and the partial pressure of oxygen in the plasma. The total content of O_2 in blood is directly related to S_{O_2} for any given Hb concentration, because the amount of O_2 that is physically dissolved in the blood is relatively small.

Arterial P_{O_2} and S_{O_2} have different physiological meanings. Arterial P_{O_2} determines the efficiency of alveolar ventilation; S_{O_2} indicates the amount of O_2 per unit of blood. It is possible to derive S_{O_2} from P_{O_2} measurements by using an ODC, but significant errors result for abnormal physiological situations unless the temperature and pH of the blood, the type of Hb derivative, and 2,3-diphosphoglycerate (DPG) are known. Direct measurement of S_{O_2} is more accurate than an indirect calculation, because the affinity of Hb for O_2 is affected by these several variables.

For young adults, the normal range of P_{O_2} in arterial blood is from 90 to 100 mm Hg (12 to 13.3 kPa). As a result of the sigmoid nature of the O_2 disassociation curve, a P_{O_2} of 60 mm Hg (8 kPa) still provides an O_2 saturation of 85%. Decreases in P_{O_2} are seen in a variety of settings. These can be divided into two groups: (1) decreased delivery of O_2 to the site of O_2 exchange between the inspired air and the blood (the lung alveoli) and (2) decreased delivery of blood to the alveoli to which O_2 is being supplied. Examples of the first group include decreased overall ventilation (such as caused by narcotic overdose or paralysis of the ventilatory muscles), obstruction of major airways

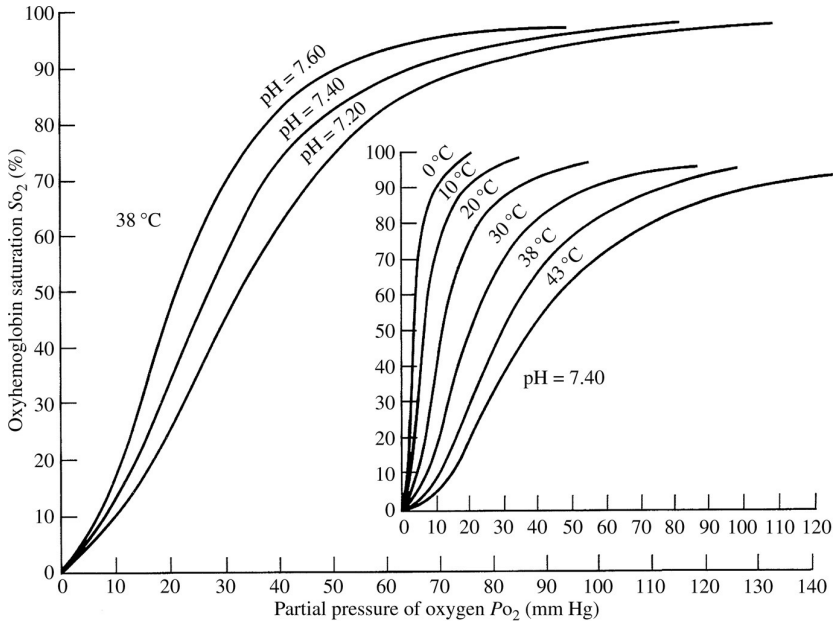


Figure 10.1 The oxyhemoglobin dissociation curve shows the effect of pH and temperature on the relationship between So_2 and Po_2.

(such as by aspirated foreign objects such as food; by spasm of the airway muscles, such as that which occurs in an acute attack of asthma); or by filling of the alveoli and small airways with fluid (such as in pneumonia or pulmonary edema). Examples of the second group include congenital cardiac abnormalities, in which blood is shunted past the lungs (the Tetralogy of Fallot, for example), and obstruction of flow through the pulmonary blood vessels (such as caused by pulmonary emboli). The important lung diseases of emphysema and chronic bronchitis usually display characteristics of both these types of abnormalities.

The P_{CO_2} level is an indicator of the adequacy of ventilation and is therefore increased in the first group of disorders discussed above, but it is generally normal in the second group unless the defect is massive in nature. In young adults, the normal range of P_{CO_2} in arterial blood is 35 to 40 mm Hg (4.7 to 5.3 kPa).

The acid–base status of the blood is assessed by measuring the hydrogen ion concentration $[H^+]$. It is conventional to use the negative logarithm to the base 10 (pH) to report this quantity; that is,

$$\text{pH} = -\log_{10}[H^+] \tag{10.2}$$

The normal range of pH in arterial blood is 7.38 to 7.44. Decreases in pH (increased quantity of hydrogen ions) occur with a decreased rate of excretion of CO_2 (respiratory acidosis) and/or with increased production of fixed acid (such as occurs in diabetic ketoacidosis) or abnormal losses of bicarbonate (the principal hydrogen ion buffer in the blood). Acidosis resulting from the last two processes is called *metabolic acidosis*. Increases in pH (decreased quantity of hydrogen ions) occur with an increased rate of excretion of CO_2 (respiratory alkalosis) and/or abnormal losses of acid (such as result from prolonged vomiting), which is called *metabolic alkalosis*. Table 10.2 gives examples of arterial-blood gases in different clinical situations. Note that a measurement of P_{CO_2}, or the level of bicarbonate in the blood, along with a measurement of pH, must be done in order to classify the type of acid–base abnormality (Davenport, 1975).

EXAMPLE 10.1 A blood specimen has a hydrogen ion concentration of 40 nmol/liter and a P_{CO_2} of 60 mm Hg. What is the pH? What type of acid–base abnormality does the patient exhibit?

ANSWER

$$\text{pH} = -\log_{10}[H^+] = -\log_{10}\left[40 \times 10^{-9}\right] \text{ mol/liter} = -[1.6 - 9.0] = 7.4.$$

So pH is in the normal range 7.38 to 7.44. However, the P_{CO_2} is 60 mm Hg, which is high compared to the normal value of 40 mm Hg. Table 10.2 shows that the patient has decreased overall ventilation.

The basic concepts of ions, electrochemical cells, and reference cells are discussed in Chapter 5. This section shows how these concepts are used to design electrodes for the measurement of pH, P_{CO_2} and P_{O_2}.

10.2 ELECTROCHEMICAL SENSORS

MEASUREMENT OF pH

The measurement of pH is accomplished by utilizing a glass electrode that generates an electric potential when solutions of differing pH are placed on the two sides of its membrane (Von Cremer, 1906). Figure 10.2 is a schematic diagram of a pH electrode.

The glass electrode is a member of the class of ion-specific electrodes that react to any extent only with a specific ion.

The approach of a hydrogen ion to the outside of the membrane causes the silicate structure of the glass to conduct a positive charge (hole) into the ionic solution inside the electrode. The Nernst equation, (4.1), applies, so the voltage across the membrane changes by 60 mV/pH unit. Because the range of physiological pH is only 0.06 pH units, the pH meter must be capable of accurately measuring changes of 0.1 mV.

Table 10.2 Examples of Arterial Blood Gases in Different Clinical Situations

Example	PCO_2, mm Hg	pH	PO_2, mm Hg	Interpretation	Likely Causes	Therapy
1	40 ± 3	7.40 ± 0.03	90 ± 5	Normal blood gas		None
2	44 ± 3	7.37 ± 0.03	88 ± 5	Normal blood gas while asleep		
3	22	7.57	106	Hyperventilation	Anxiety	None
4	68	7.10	58	Hypoventilation	Central nervous system depression; blockage of upper airway	Mechanical ventilation; relieve the cause
5	58	7.21	39	Hypoventilation and hypoxemia	Pneumonia; small-airway obstruction; severe asthma	Oxygen; bronchodilators; mechanical ventilation
6	61	6.99	29	Combined respiratory and metabolic acidosis and hypoxemia	Birth asphyxia; near-drowning	Oxygen; mechanical ventilation; buffers?
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	Treat chronic disease; no additional therapy may be necessary
8	29	7.31	106	Metabolic acidosis with respiratory compensation	Diabetic; ketoacidosis; dehydration	Treat the cause; buffers?

SOURCE: B. G. Nickerson and F. Monaco, "Carbon dioxide electrodes, arterial and transcutaneous," in J. G. Webster (ed.), Encyclopedia of Medical Devices and Instrumentation. New York: Wiley, 1988, pp. 564–569.

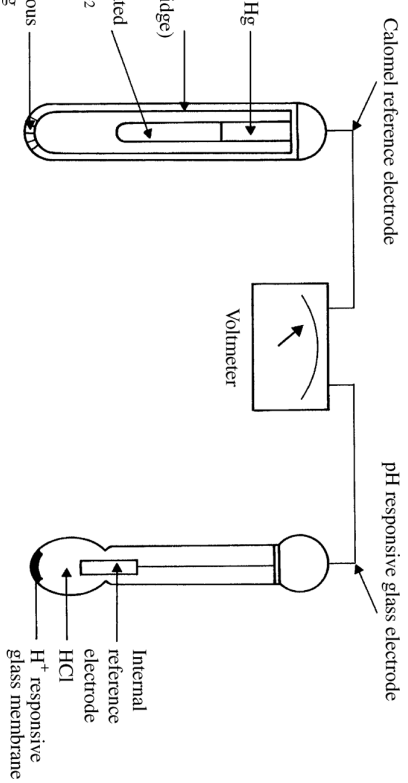


Figure 10.2 pH electrode (From R. Hicks, J. R. Schenken, and M. A. Steinrauf, Laboratory Instrumentation. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

The basic approach is to place a solution of known pH on the inside of the membrane and the unknown solution on the outside. Hydrochloric acid is generally used as the solution of known pH. A reference electrode, usually an Ag/AgCl or a saturated calomel electrode, is placed in this solution. A second reference electrode is placed in the specimen chamber. A salt bridge is included within the reference to prevent the chemical constituents of the specimen from affecting the voltage of the reference electrode. The potential developed across the membrane of the glass electrode is read by a pH meter. This pH meter must have extremely high input impedance, because the internal impedance of the pH electrode is in the 10 to 100 MΩ range.

EXAMPLE 10.2 Design an amplifier for use with the pH electrode. An output in the range of 1 to 2 mV is desired for the normal pH variation of blood.

ANSWER Because the internal impedance of the pH electrode is in the 10 to 100 MΩ range, we need an amplifier with extremely high input impedance and extremely small bias current. Thus, select a field-effect transistor (FET) op amp that has specifications for extremely low bias current and extremely low offset voltage drift. To achieve high input impedance, connect it as a noninverting amplifier with a gain of 101.

The Nernst equation shows that the voltage produced by a pH electrode varies with the temperature of the specimen and the reference solution. Some pH electrodes include a water bath that allows the pH determination to be made at 37 °C; others require a temperature correction. This temperature correction can be made by changing the constant used to convert from the electrode voltage to the meter scale reading in pH units by setting a temperature-control knob to the temperature at which the pH measurement is being made.

A more complex correction includes the effect on instrument output of temperature and the CO_2 content of the specimen (Adamsons *et al.*, 1964; Burton, 1965). This type of correction can be made in modern devices that measure pH and P_{CO_2} and also have memory and computational capabilities.

Calibration with solutions of known pH is performed before measurements of patient specimens are made. Two solutions are normally used: one with a pH near 6.8 and one with the pH near 7.9.

MEASUREMENT OF P_{CO_2}

The measurement of P_{CO_2} is based on the fact that the relationship between log P_{CO_2} and pH is linear over the range of 10 to 90 mm Hg (1.3 to 12 kPa), which includes essentially all the values of clinical interest. This result can be established by examining some fundamental chemical relationships among H^+, H_2CO_3, HCO_3^-, and P_{CO_2}. The first three quantities are related by the equilibrium equation

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \tag{10.3}$$

In addition, the relationship between P_{CO_2} and the concentration of CO_2 dissolved in the blood, $[CO_2]$, is given by

$$[CO_2] = a(P_{CO_2}) \tag{10.4}$$

where $a = 0.0301$ mmol/liter per mm Hg P_{CO_2}. The mass relationship corresponding to (10.3) can then be written as

$$k' = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \tag{10.5}$$

Next we use the fact that $[H_2CO_3]$ is proportional to $[CO_2]$ to obtain the result

$$k = \frac{[H^+][HCO_3^-]}{[CO_2]} \tag{10.6}$$

where k represents the combined values of k' and the proportionality constant between $[H_2CO_3]$ and $[CO_2]$. Now, using (10.4), we obtain the following result:

$$k = \frac{[H^+][HCO_3^-]}{aP_{CO_2}} \tag{10.7}$$

Next, taking the base-10 logarithm of (10.7) and rearranging, we obtain

$$\log[H^+] + \log[HCO_3^-] - \log k - \log a - \log P_{CO_2} = 0 \tag{10.8}$$

Using the definition of pH yields

$$\text{pH} = \log[HCO_3^-] - \log k - \log a - \log P_{CO_2} \tag{10.9}$$

This shows that pH has a linear dependence on the negative of log P_{CO_2}.

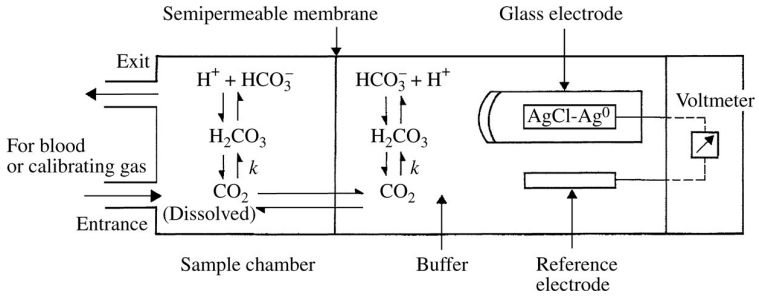


Figure 10.3 **P_{CO_2} electrode** (From R. Hicks, J. R. Schenken, and M. A. Steinrauf, Laboratory Instrumentation. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

This result is used in the construction of the P_{CO_2} electrode shown in Figure 10.3 (Severinghaus, 1965). The assembly includes two chambers, one for the specimen and a second containing a pH electrode of the type discussed. In contrast to the basic pH-measurement device in which the pH electrode is placed in the specimen, in this case the pH electrode is bathed by a buffer solution of bicarbonate and NaCl.

The two chambers are separated by a semipermeable membrane, usually made of Teflon or silicone rubber. This membrane allows dissolved CO_2 to pass through but blocks the passage of charged particles, in particular H^+ and HCO_3^-. When the specimen is placed in its chamber, CO_2 diffuses across the membrane to establish the same concentration in both chambers. If there is a net movement of CO_2 into (or out of) the chamber containing the buffer, $[H^+]$ increases (or decreases), and the pH meter detects this change. Because the relationship between pH and the negative log P_{CO_2} is only a proportional one, it is necessary to calibrate the instrument before each use with two gases of known P_{CO_2}.

Using the values of pH obtained by processing these two standards, we obtain a calibration curve of P_{CO_2} versus pH. We then use the measured pH value to obtain the specimen's P_{CO_2} from this curve. With some instruments, the capability of calibrating the P_{CO_2} electrode is built into the instrument so that the calibration curve is set up in the electronics of the instrument by setting the values of two potentiometers.

THE P_{O_2} ELECTRODE

Figure 10.4 shows the basic components of the Clark-type polarographic electrode. The measurement of P_{O_2} is based on the following reactions. At the cathode, reduction occurs:

$$O_2 + 2H_2O + 4e^- \rightarrow 2H_2O_2 + 4e^- \rightarrow 4OH^-$$
$$4OH^- + 4KCl \rightarrow 4KOH + 4Cl^- \tag{10.10}$$

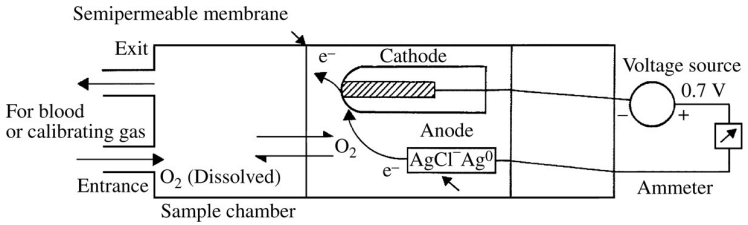


Figure 10.4 P_{O_2} **electrode** (From R. Hicks, J. R. Schenken, and M. A. Steinrauf, Laboratory Instrumentation. Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

The hydroxyl ions created in this reaction are buffered by the electrolyte. At the anode, which in this P_{O_2} electrode is the reference electrode, oxidation occurs.

$$4Ag + 4Cl^- \rightarrow 4AgCl + 4e^- \tag{10.11}$$

This produces the four electrons required for the reaction in (10.10).

The cathode is constructed of glass-coated Pt, and the reference electrode is made of Ag/AgCl.

The plot of current versus polarizing voltage of a typical P_{O_2} electrode (polarogram) is shown in Figure 10.5(a). The polarizing voltage is selected in the ''plateau'' region to provide a sufficient potential to drive the reaction, without permitting other electrochemical reactions that would be driven by greater voltages to take place. Thus the resulting current is linearly proportional to the number of O_2 molecules in solution [see Figure 10.5(b)]. The O_2 membrane is permeable to O_2 and other gases and separates the electrode from its surroundings.

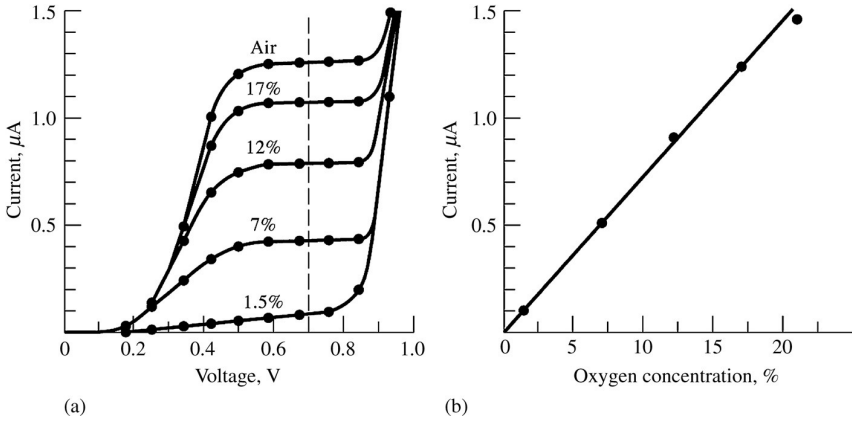
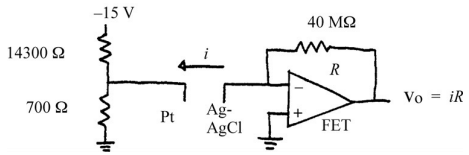


Figure 10.5 (a) Current plotted against polarizing voltage for a typical P_{O_2} electrode for the percents O_2 shown. (b) Electrode operation with a polarizing voltage of 0.68 V gives a linear relationship between current output and percent O_2.

A polarizing voltage of 600 to 800 mV is required for these reactions to occur. This voltage is usually supplied by a mercury cell.

We determine the value of P_{O_2} by using the fact that the flow of current through the external circuit connecting the electrodes is proportional to P_{O_2}. The presence of O_2 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 P_{O_2} level. When the P_{O_2} level is zero, the current flowing through the circuit is called the background current. Part of the calibration sequence involves setting the P_{O_2} meter to zero when a CO_2/N_2 gas is bubbled through the specimen chamber. Slow bubbling is used to ensure proper temperature equilibration.

EXAMPLE 10.3 Design an amplifier and a power source for an O_2 electrode. The output of your device should range from 0 to 10 V for an oxygen range from 0% to 100%. At a 20% O_2 level, the electrode current is 50 nA.



ANSWER From a −15 V power supply use a 14,300 Ω and 700 Ω resistor voltage divider to yield −0.7 V to bias the Pt electrode. Feed the Ag/AgCl electrode output into an FET current-to-voltage converter with a feedback resistor $= V/I = 10\,\text{V}/250\,\text{nA} = 40\,\text{M}\Omega$.

Equation (10.10) shows that the reaction consumes O_2. This loss is a direct function of the area of the Pt electrode that is exposed to the reaction solution and the permeability of the semipermeable membrane to O_2. The exposed area of the Pt electrode usually has a diameter of 20 μm.

The choice of the semipermeable membrane is based on a trade-off between consumption of O_2 and the time required for the P_{O_2} values in the specimen and measurement chambers to equilibrate. The more permeable the membrane is to O_2, the higher the consumption of O_2 and the faster the response. Polypropylene is less permeable than Teflon and is preferable in most applications. Polypropylene is also quite durable, and it maintains its position over the electrode more reliably than other membrane materials.

The membrane thickness and composition determine the O_2 diffusion rate; thicker membranes extend the sensor time response by significantly increasing the diffusion time and produce smaller currents.

Because the electrode consumes O_2, it partially depletes the oxygen in the immediate vicinity of the membrane. If movement of the sample takes place, undepleted solution brought to the membrane causes a higher instrument

reading—the ''stirring'' artifact. This is avoided by waiting for a stagnant equilibrium to occur.

The reaction is very sensitive to temperature. To maintain a linear relationship between P_{O_2} and current, the temperature of the electrode must be controlled to ± 0.1 °C. This has been traditionally accomplished by using a water jacket. However, new blood-gas analyzers are now available that use precision electronic heat sources. The current through the meter is approximately 10 nA/mm Hg (75 nA/kPa) O_2 at 37 °C, so the instruments must be designed to be accurate at very low current levels.

The system is calibrated by using two gases of known O_2 concentration. One gas with no O_2 (typically a CO_2-N_2 mixture) and a second with a known O_2 content (usually an $O_2-CO_2-N_2$ mixture) are used. The specimen chamber is filled with water, and the calibrating gas containing no O_2 is bubbled through it. The P_{O_2} meter output is set to zero after equilibrium of O_2 content is achieved—usually in about 90 s. Next the second calibrating gas is used to determine the second point on the P_{O_2}-versus-electrode-current calibration scale, which is electrically set in the machine. Then the value of the specimen P_{O_2} can be measured. Note that the time required to reach equilibrium is a function of the P_{O_2} of the specimen. It may take as long as 360 s for a specimen with a P_{O_2} of 430 mm Hg (57 kPa) to reach equilibrium (Moran *et al.*, 1966). Drägerwerk Aktiengesellschaft, Lübeck, Germany, manufactures a gas O_2 sensor with 2 s response in which gas diffuses into a P_{O_2} electrode.

11.1 SPECTROPHOTOMETRY

Spectrophotometry is the basis for many of the instruments used in clinical chemistry. The primary reasons for this are ease of measurement, satisfactory accuracy and precision, and the suitability of spectrophotometric techniques to use in automated instruments. In this section, *spectrophotometer* is used as a general term for a class of instruments. Photometers and colorimeters are members of this class (Shen *et al.*, 2006).

Spectrophotometry is based on the fact that substances of clinical interest selectively absorb or emit electromagnetic energy at different wavelengths. For most laboratory applications, wavelengths in the range of the ultraviolet (200 to 400 nm), the visible (400 to 700 nm), or the near infrared (700 to 800 nm) are used; the majority of the instruments operate in the visible range.

Figure 11.1 is a general block diagram for a spectrophotometer-type instrument. The source supplies the radiant energy used to analyze the sample. The wavelength selector allows energy in a limited wavelength band to pass through. The cuvette holds the sample to be analyzed in the path of the energy. The detector produces an electric output that is proportional to the amount of energy it receives, and the readout device indicates the received energy or some function of it (such as the concentration, in the sample, of a substance of interest).

The basic principle of a spectrophotometer is that if we examine an appropriately chosen, sufficiently small portion of the electromagnetic spectrum, we can use the energy-absorption properties of a substance of interest to measure the concentration of that substance. In the vast majority of cases, these substances, as they are normally found in a patient's samples (of serum,

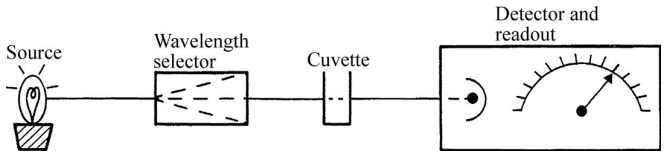


Figure 11.1 Block diagrams of a spectrophotometer (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry,* 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

urine, or CSF, for instance), do *not* exhibit the desired energy-absorption characteristics. In such cases, reagents are added to the sample, causing a reaction to occur. This reaction yields a product that *does* have the desired characteristics. The reaction products are then placed in the cuvette for analysis. The instrument-calibration procedures take into account the possible difference in concentration between the reaction product and the original quantity of interest.

Let us discuss in detail the characteristics of each of the subsystems shown in Figure 11.1.

Power Sources Hydrogen or deuterium discharge lamps are used to provide power in the 200-to-360 nm range, and tungsten filament lamps are used for the 360-to-800 nm range. Hydrogen and deuterium lamps both produce a continuous spectrum; but a problem with these power sources is that they produce about 90% of their power in the infrared range. The output in the ultraviolet and visible ranges can be increased by operating the lamp at voltages above the rated value, but this stratagem significantly reduces the expected life of the lamp. Another problem with tungsten lamps is that, during operation, the tungsten progressively vaporizes from the filaments and condenses on the glass envelope. This coating, which is generally uneven, alters the spectral characteristics of the lamp and can cause errors in determinations.

Wavelength Selectors A variety of devices are used to select those portions of the power spectrum produced by the power source that are to be used to analyze the sample. These devices can be divided into two classes: filters and monochromators. There are two basic types of filters: glass filters and interference filters.

Glass filters function by absorbing power. For example, a blue-colored filter absorbs in the higher-wavelength visible range (red region) and transmits in the lower-wavelength visible range (blue-green region). These filters (consisting of one or more layers of glass plates) are designed to be low-pass, high-pass, or bandpass (a combination of low- and high -pass) filters.

Interference filters are made by spacing reflecting surfaces such that the incident light is reflected back and forth a short distance. The distance is selected such that light in the wavelength band of interest tends to be in phase and to be reinforced; light outside this band is out of phase and is canceled (the interference effect). Harmonics of the frequencies in this band are also passed and must be eliminated by glass cutoff filters.

Glass filters are used in applications in which only modest accuracy is required. Interference filters are used in many spectrophotometers, including those used in the SMAC (Technicon Instrument Corporation) and the CentrifiChem (Union Carbide). Devices that use filters as their wavelength selectors are called colorimeters or photometers.

Monochromators are devices that utilize prisms and diffraction gratings. They provide very narrow bandwidths and have adjustable nominal wavelengths. The basic principle of operation of these devices is that they disperse the input beam spatially as a function of wavelength. A mechanical device is then used to allow wavelengths in the band of interest to pass through a slit.

Prisms are constructed from glass and quartz. Quartz is required for wavelengths below 350 nm. A convergent lens system is used to direct the light from the source through an entrance slit. The prism bends the light as a function of wavelength. The smaller wavelengths (ultraviolet) are bent the most. This produces an output beam in which the wavelength band of interest can be selectively passed by placing in the light path an opaque substance with a slit in it. The wavelength spectrum of the power passing through the slit is nominally triangle shaped. In prisms, as in filters, the wavelength at which maximal transmittance occurs is the nominal central wavelength. Bandwidths of 0.5 nm can be obtained with this type of device. Prisms have been used over the wavelength range of 220 to 950 nm. The nonlinear spatial distribution of the power emerging from a prism requires relatively complex mechanical devices for control of the slit position to select different nominal wavelengths.

Diffraction gratings are constructed by inscribing a large number of closely spaced parallel lines on glass or metal. A grating exploits the fact that rays of light bend around sharp corners. The degree of bending is a function of wavelength. This results in separation of the light into a spectrum at each line. As these wave fronts move and interact, reinforcement and cancellation occur. The light emerging from a grating is resolved spatially in a linear fashion, unlike the light from a prism, in which the separation of wavelengths is less at longer wavelengths. As in the case of the prism, a slit is used to select the desired bandwidth. The mechanics of the slit-positioning mechanism of a grating are less complicated than those of a prism because of the linearity of the spatial separation of the wavelengths. Gratings can achieve bandwidths down to 0.5 nm and can operate over the range of 200 to 800 nm.

Cuvette The cuvette (Figure 11.1) holds the substance being analyzed. Its optical characteristics must be such that it does not significantly alter the spectral characteristics of the light as that light enters or leaves the cuvette. The degree of care and expense involved in cuvette design is a function of the overall accuracy required of the spectrophotometer.

Sample The sample (actually, in most cases, the substances resulting from the interaction of the patient specimen and appropriate reagents) absorbs light

selectively according to the laws of Lambert, Bouguer, Bunsen, Roscoe, and Beer. The principles stated in these laws are usually grouped together and called Beer's law. The essence of the law was stated by Bouguer: ''Equal thickness of an absorbing material will absorb a constant fraction of the energy incident upon it.'' This relationship can be stated formally as follows:

$$P = P_0 10^{-aLC} \tag{11.1}$$

where

P_0 = radiant power arriving at the cuvette
P = radiant power leaving the cuvette
a = absorptivity of the sample (extinction coefficient)
L = length of the path through the sample
C = concentration of the absorbing substance

Absorptivity is a function of the characteristics of the sample and the wavelength content of the incident light. This relationship is often rewritten in the form

$$\%T = 100P/P_0 = (100)10^{-aLC} \tag{11.2}$$

where $\%T$ is the percent transmittance. The value of a is constant for a particular unknown, and the cuvette and cuvette holder are designed to keep L as nearly constant as possible. Therefore, changes in P should reflect changes in the concentration of the absorbing substance in the sample.

Percent transmittance is often reported as the result of the determination. However, because the relationship between concentration and percent transmittance is logarithmic, it has been found convenient to report absorbance. Absorbance A is defined as $\log (P_0/P)$, so

$$A = \log\left(\frac{P_0}{P}\right) = \log\left(\frac{100}{\%T}\right) = 2 - \log(\%T) \tag{11.3}$$

Note that the relationship

$$A = aLC \tag{11.4}$$

follows from (11.1) and (11.3). As previously stated, the spectrophotometer is designed to keep a and L as nearly constant as possible so that a particular determination A ideally varies only with C. Therefore, the concentration of an unknown can be determined as follows. The absorbance A_s of a standard with known concentration of the substance of interest, C_s, is determined. Next the absorbance of the unknown, A_u, is determined. Finally, the concentration of the unknown, C_u, is computed via the relationship

$$C_u = C_s\left(\frac{A_u}{A_s}\right) \tag{11.5}$$

If this relationship holds over the possible range of concentration of the unknown substance in patient samples, then the determination is said to obey Beer's law. This relationship may not hold, however, because of absorption by the solvent or reflections at the cuvette. Then a relatively large number of standards with concentration values spanning the range of interest must be used to compute a calibration curve of concentration versus absorbance. This curve is then employed to obtain a concentration value for the absorbance value of the unknown.

EXAMPLE 11.1 A filter photometer is being used to determine total concentration of serum protein (grams per deciliter). A technologist runs one standard with a known total protein concentration of 8 g/dl and obtains a $\%T$ reading of 20%, processes a patient sample and gets a $\%T$ reading of 30%, assumes the instrument's operation satisfies Beer's law, and calculates the patient value. What value should be obtained? Do you agree with the methodology? If not, what would you do differently and why?

ANSWER From (11.3), $A_s = 2 - \log \%T = 2 - \log 20 = 2 - 1.30 = 0.70$. $A_u = 2 - \log 30 = 2 - 1.48 = 0.52$. From (11.5)

$$C_u = C_s\frac{A_s}{A_u} = 8\frac{0.52}{0.70} = 5.9\ \text{g/dl}$$

Do not agree with the methodology. Because of absorption by the solvent or reflections at the cuvette, Beer's law may not hold. Run more than one standard of known concentration to ensure that Beer's law holds.

EXAMPLE 11.2 A spectrophotometer is being calibrated before being used to determine concentration of serum calcium. Four standards (samples of known calcium concentration) are analyzed, and the following values emerge.

Standards	% Transmittance	Calcium Concentration, mg/dl
1	79.4	2
2	39.8	8
3	31.6	10
4	20.0	14

Does this determination follow Beer's law? If a patient sample was processed and a percentage of 35 were obtained, what would the calcium concentration be?

ANSWER

$$A_1 = 2 - \log 79.4 = 2 - 1.90 = 0.10$$
$$A_2 = 2 - \log 39.8 = 2 - 1.60 = 0.40$$
$$A_3 = 2 - \log 31.6 = 2 - 1.50 = 0.50$$
$$A_4 = 2 - \log 20 = 2 - 1.30 = 0.70$$

For all four samples ratio of concentration to Absorbance is given as:

$$\frac{2}{0.1} = \frac{8}{0.4} = \frac{10}{0.5} = \frac{14}{0.7} = 20$$

Therefore, the samples follow Beer's law. For $\%T = 35$,

1. Absorbance $= 2 - \log(\%T) = 2 - \log 35 = 0.46$.
2. Concentration is given as $C_u = C_s(A_u/A_s) = 2(0.46/0.1) = 9.1$ mg/dl.

The amount of light absorbed by a compound is generally a function of wavelength. The chemical reaction used in preparing the sample for spectrophotometry is designed to produce a compound (1) the concentration of which is proportional to that of the compound of interest and (2) the peak of the absorption spectrum of which is separated from the absorption peaks of the other compounds in the sample.

The wavelength band of light allowed to pass through the wavelength selector is generally chosen to cover the peak of the absorption curve symmetrically. There are a number of other factors to consider, however, including the absolute level of absorbance at the peak and its wavelength value. If the absorbance is too great ($A > 1.0$) or too small ($A < 0.11$), the errors of the photometric system become unacceptably large (Henry, 1984). In the case in which the absorbance is very large, the sample can be diluted, but this procedure is time-consuming and can result in errors. The wavelength of the peak must be within the range of the spectrophotometer's capabilities.

10.3 CHEMICAL FIBROSENSORS

Rapid advances in the communications industry have provided appropriate small optical fibers, high-energy sources such as lasers, and wavelength detectors. The fiber-optic sensors that were developed were called *optodes*, a term coined by Lübbers and Opitz (1975), which implies that optical sensors are very similar to electrodes. As we shall see, however, the properties and operating principles for optical fibrosensors are quite different from those for electrodes. The term *optrode*, with an *r*, is currently used.

Chemical fibrosensors offer several desirable features.

1. They can be made small in size.
2. Multiple sensors can be introduced together, through a catheter, for intracranial or intravascular measurements.
3. Because optical measurements are being made, there are no electric hazards to the patient.
4. The measurements are immune to external electric interference, provided that the electronic instrumentation is properly shielded.
5. No reference electrode is necessary.

In addition, fibrosensors have a high degree of flexibility and good thermal stability, and low-cost manufacturing and disposable usage are possible. In reversible sensors, the reagent phase is not consumed by its reaction with the analyte. In nonreversible sensors, the reagent phase is consumed. The consumption of the reagent phase for nonreversible sensors must be small, or there must be a way to replenish the reagent.

Optical-fiber sensors have several limitations when compared with electrode sensors. Optical sensors are sensitive to ambient light, so they must be used in a dark environment or must be optically shielded via opaque materials. The optical signal may also have to be modulated in order to code it and make it distinguishable from the ambient light. The dynamic response of optical sensors is normally limited compared with that of electrodes. Reversible indicator sensors are based on an equilibrium measurement rather than a diffusion-dependent one, so they are less susceptible to changes in flow concentration at the sensor (Seitz, 1988).

Long-term stability for optical sensors may be a problem for reagent-based systems. However, this can be compensated for by the use of multiple-wavelength detection and by the ease of changing reagent phases. In addition, because the reagent and the analyte are in different phases, a mass-transfer step is necessary before constant response is achieved (Seitz, 1988). This limits the temporal response of an optical sensor. Another consideration with optical

sensors is that for several types of optical sensors, the response is proportional to the amount of reagent phase. For small amounts of reagent, an increased response can be achieved by increasing the intensity of the source. An increased response, however, results in an increase in the photodegradation process of the reagent. Designers of optical sensors, then, must consider amount of the reagent phase, intensity of the light source, and system stability (Seitz, 1984).

These limitations can be alleviated by an appropriate design of the optical sensor and instrumentation system (Wise, 1990). The systems described in the following paragraphs incorporate many features specifically for this purpose.

INTRAVASCULAR MEASUREMENTS OF OXYGEN SATURATION

Blood oxygen can be monitored by means of an intravascular fiber-optic catheter. These catheters are used to monitor mixed venous oxygen saturation during cardiac surgery and in the intensive-care unit. A Swan–Ganz catheter is used (see Section 7.11), in which a flow-directed fiber-optic catheter is placed into the right jugular vein. The catheter is advanced until its distal tip is in the right atrium, at which time the balloon is inflated. The rapid flow of blood carries the catheter into the pulmonary artery.

Measurements of mixed venous oxygen saturation give an indication of the effectiveness of a cardiopulmonary system. Measurements of high oxygen saturation in the right side of the heart may indicate congenital abnormalities of the heart and major vessels or the inability of tissue to metabolize oxygen. Low saturation readings on the left side of the heart may indicate a reduced ability of the lungs to oxygenate the blood or of the cardiopulmonary system to deliver oxygen from the lungs. Low saturation readings in the arterial system indicate a compromised cardiac output or reduced oxygen-carrying capacity of the blood.

Figure 10.6 shows the optical-absorption spectra for oxyhemoglobin, carboxyhemoglobin, hemoglobin, and methemoglobin. Measurements in the red region are possible because the absorption coefficient of blood at these wavelengths is sufficiently low that light can be transmitted through whole blood over distances such that feasible measurements can be made with fiber-optic catheters. Note that the 805 nm wavelength provides a measurement independent of the degree of oxygenation. This isosbestic wavelength is used to compensate for the scattering properties of the whole blood and to normalize the measurement signal with any changes in hemoglobin from patient to patient.

Oxygen saturation is measured by taking the ratio of the diffusely back-scattered light intensities at two wavelengths. The first wavelength is in the red region (660 nm); the second is in the infrared region (805 nm), which is known as the isosbestic point for Hb and HbO_2. Oxygen saturation is given by (10.1), which considers the optical density of the blood—the light

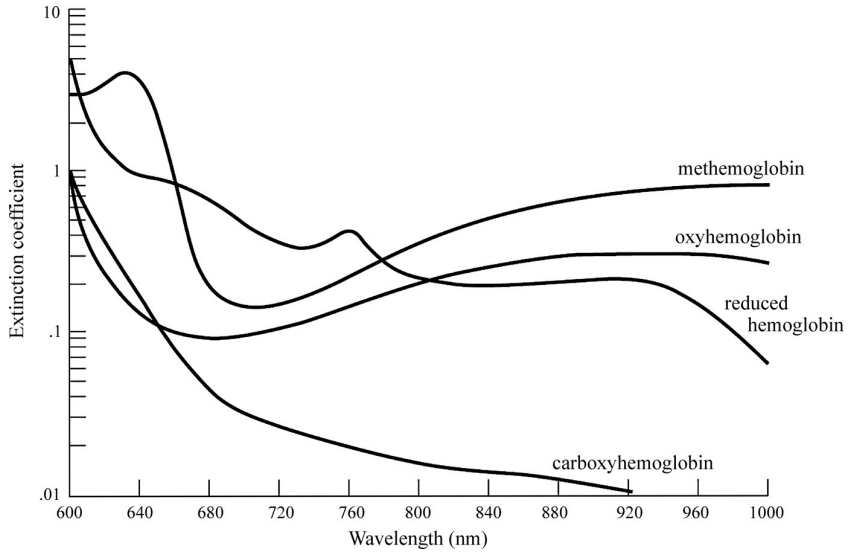


Figure 10.6 Absorptivities (extinction coefficients) in liter/(mmol·cm) of the four most common hemoglobin species at the wavelengths of interest in pulse oximetry. (Courtesy of Susan Manson, Biox/Ohmeda, Boulder, CO.)

transmitted through the blood—according to Beer's law. For hemolyzed blood (blood with red cells ruptured), Beer's law (Section 11.1) holds, and the absorbance (optical density) at any wavelength is (Allan, 1973)

$$A(\lambda) = WL[a_o(\lambda)C_o + a_r(\lambda)C_r] \tag{10.12}$$

where

W = weight of homoglobin per unit volume
L = optical path length
a_o and a_r = absorptivities of HbO_2 and Hb
$C_o = C_r$ = relative concentrations of HbO_2 and Hb ($C_o + C_r = 1.0$)

Figure 10.6 shows that a_o and a_r are equal at 805 nm, called the *isosbestic* wavelength. If this wavelength is λ_2, then

$$WL = \frac{A(\lambda_2)}{a(\lambda_2)} \tag{10.13}$$

where

$$a(\lambda_2) = a_o(\lambda_2) = a_r(\lambda_2) \tag{10.14}$$

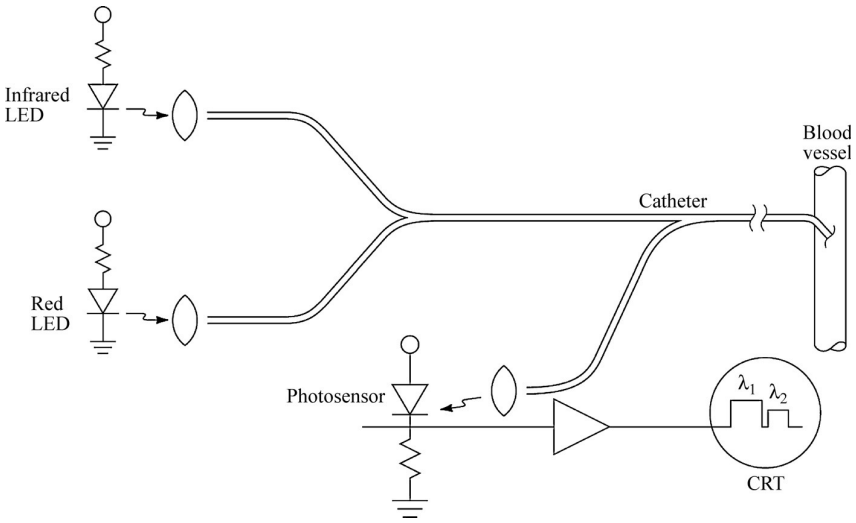


Figure 10.7 The oximeter catheter system measures oxygen saturation *in vivo*, using red and infrared light-emitting diodes (LEDs) and a photosensor. The red and infrared LEDs are alternately pulsed in order to use a single photosensor.

Therefore,

$$A(\lambda) = \frac{A(\lambda_2)}{a(\lambda_2)}[a_o(\lambda)C_o + a_r(\lambda)C_r] \tag{10.15}$$

When absorbance is measured at a second wavelength λ_1, the oxygen saturation is given by

$$C_o = x + \frac{yA(\lambda_1)}{A(\lambda_2)} \tag{10.16}$$

where x and y are constants that depend only on the optical characteristics of blood. In practice, λ_1 is chosen to be that wavelength at which the difference between a_o and a_r is a maximum, which occurs at 660 nm [see Figure 10.6].

Figure 10.7 shows a fiber-optic instrument devised to measure oxygen saturation in the blood. This device, which could also be used for measuring cardiac output with a dye injected, is described here. The instrument consists of red and infrared light-emitting diodes (LEDs) and a photosensor. Plastic optical fibers are well adapted to these wavelengths. Figure 10.8 shows a fiber-optic oximeter catheter that is flow directed. After insertion, the balloon is inflated, and blood flow drags the tip through the chambers of the heart.

In addition to measuring blood-oxygen saturation through reflectance, the same dual-wavelength optics can be used to measure blood flow by dye dilution. Indo/cyanine/green, which absorbs light at 805 nm (the isosbestic wavelength of oxyhemoglobin), is used as the indicator. This is a dual-fiber system. Light at 805 nm is emitted from one fiber, scattered by the blood cells,

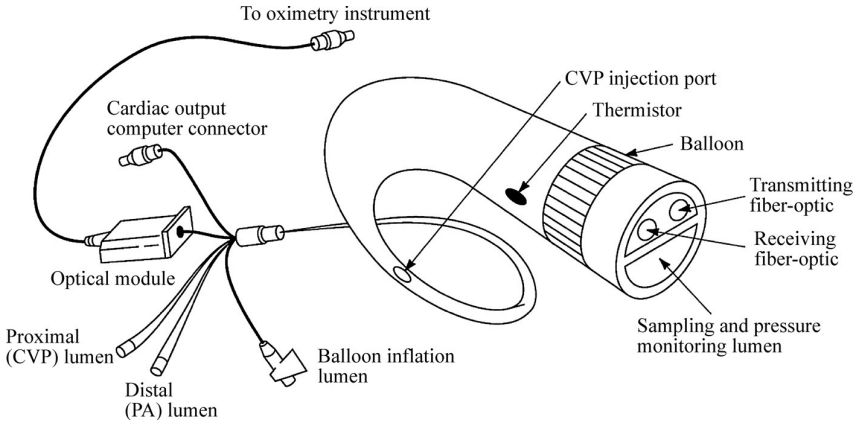


Figure 10.8 The catheter used with the Abbott Opticath Oximetry System transmits light to the blood through a transmitting optical fiber and returns the reflected light through a receiving optical fiber. The catheter is optically connected to the oximetry processor through the optical module. (From Abbott Critical Care Systems. Used by permission.)

attenuated by the dye in the blood, and partially collected by the other fiber for measurement. The second wavelength, above 900 nm, is used as a reference; this is the region where the light is absorbed by the dye. It is used to compare the effect of flow-rate light scattering. In effect, a dual-beam ratiometric system is developed for dye-dilution measurements of blood flow. Cardiac output is determined via the dye-dilution method described in Section 8.2.

A significant difference exists between two-wavelength oximetry systems and the Abbott three-wavelength Oximetry Opticath System. In two-wavelength systems an important limitation, in the *in vivo* measurement of oxygen saturation below 80%, is the dependence of the reflected light's intensity on the patient's hematocrit. Hematocrit varies from subject to subject, and within one subject it varies for different physiological conditions. Catheter tip oximeters require frequent updates of a patient's hematocrit. Various correction techniques have been devised to correct the oxygen-saturation measurements for errors due to hematocrit variations. (This limitation is eliminated in the three-wavelength Abbott Opticath Oximetry System.) False readings occur in situations in which hemoglobin combines with another substance besides oxygen, such as carbon monoxide. Hemoglobin has a strong affinity for carbon monoxide, so oxygen is displaced. The optical spectra for HbO_2 and HbCO overlap at 660 nm (Figure 10.6), causing an error in So_2 if CO is present in the blood.

A three-fiber intravascular fiber-optic catheter that measures mixed venous oxygen saturation and hematocrit simultaneously has been developed and tested (Mendelson *et al.*, 1990). The system consists of a catheter with a single light source in two equally spaced, near and far detecting fibers. The

ratio of backscattered-light intensities measured at the isosbestic wavelength (805 nm) by the two detecting fibers (IR near/IR far) serves a correction factor that reduces the dependence of oxygen-saturation measurements on hematocrit.

This approach also provides a means for determining hematocrit independently. The principle of the measurement is based on the fact that variations in blood pH and osmolarity affect the shape and volume of the red blood cells. The IR near/IR far ratio is affected by variations in red blood cell volume and thus in hematocrit. The reflected-light intensities, measured by the two detecting fibers, are due to the higher-order multiple scattering. The intensity of the reflected light becomes more pronounced as source-to-detector separation distance increases. Details concerning the transcutaneous measurement of arterial oxygen saturation via pulse oximetry are given in Section 10.6.

REVERSIBLE-DYE OPTICAL MEASUREMENT OF pH

The continuous monitoring of blood pH is essential for the proper treatment of patients who have metabolic and respiratory problems. Small pH probes have been developed for intravascular measurement of the pH of the blood (Peterson *et al.*, 1980). These instruments require a range of 7.0 to 7.6 pH units and a resolution of 0.01 pH unit.

Figure 10.9 shows an early version of a pH sensor, in which a reversible colorimetric indicator system is fixed inside an ion-permeable envelope at the distal tip of the two plastic optical fibers. Light-scattering microspheres are mixed with the indicator dye inside the ion-permeable envelope in order to optimize the backscattering of light to the collection fiber that leads to the detector.

The reversible indicator dye, phenol red, is a typical pH-sensitive dye. The dye exists in two tautomeric (having different isomers) forms, depending on whether it is in an acidic or a basic solution. The two forms have different optical spectra. In Figure 10.10, the absorbance is plotted against wavelength for phenol red for the base form of the dye, indicating that the optical-

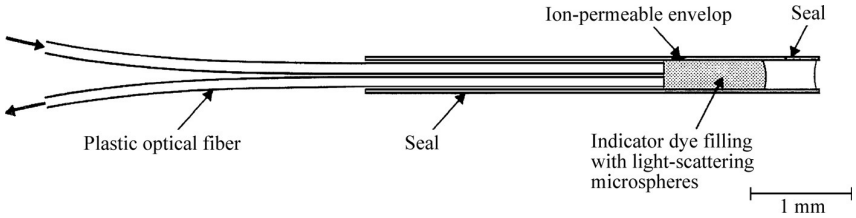


Figure 10.9 A reversible fiber-optic chemical sensor measures light scattered from phenol red indicator dye to yield pH. [From J. I. Peterson, ''Optical sensors,'' in J. G. Webster (ed.), Encyclopedia of Medical Devices and Instrumentation. New York: Wiley, 1988, pp. 2121–2133. Used by permission.]

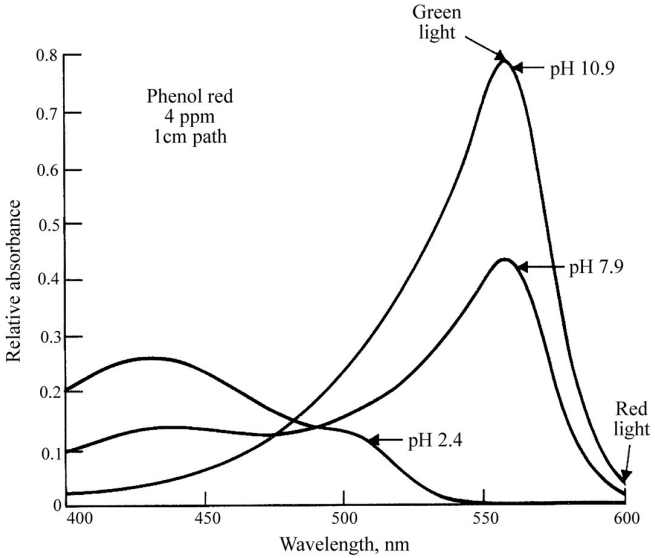


Figure 10.10 The plot of absorbance against wavelength of phenol red (base form) increases with pH for green light but is constant for red light.

absorbance peak increases with increasing pH. The ratio of green to red light transmitted through the dye is (Peterson, 1988)

$$R = k \times 10^{\left[-C/\left(10^{-\Delta}+1\right)\right]} \tag{10.17}$$

where

Δ = difference between pH and pK of the dye

$R = I(\text{green})/I(\text{red})$ = measured ratio of light intensities

$k = I_0(\text{green})/I_0(\text{red})$ = a constant (I_0 = initial light intensity)

C = a constant determined by (1) the probe geometry, (2) the total dye concentration, and (3) the absorption coefficient of the dye's basic tautomer

Equation (10.17) shows that the ratio of green to red light transmitted through the dye can be expressed as a function of (1) the ionization constant of the dye—that is, the pK_a where ''a'' indicates the dye is a weak acid; (2) Beer's law for optical absorption; and (3) the use of the definition of pH. The constants are k, the optical constant; A, the absorbance of the probe when the dye is completely in the base form; and pK, the inverse log of the ionization constant of the dye. The ratio of green to red light is used because the green light transmitted varies with pH, whereas the red light is an isosbestic wavelength and does not vary with pH. In effect, this system is a dual-beam spectrometer.

Figure 10.11 shows a plot of R, the ratio of green to red light, against Δ, the deviation of the pH from the pK of the dye. The curve shows that over a range

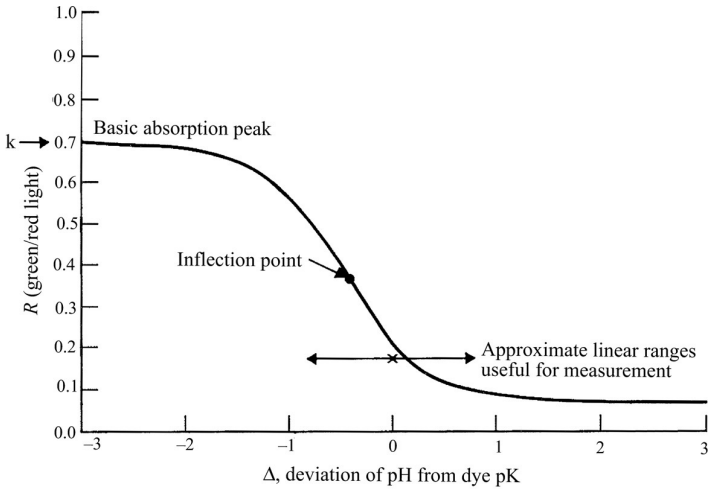


Figure 10.11 The ratio (R) of green to red light transmitted through phenol red for basic and acidic forms of the dye. Δ =deviation of pH from dye pK. (From J. I. Peterson, S. R. Goldstein, and R. V. Fitzgerald, "Fiber-optic pH probe for physiological use."*Anal. Chem.*, 1980, 52, 864–869. Used by permission.)

of about 1 pH unit, a nearly linear region for the S-shaped curve results. The instrument for pH measurement via the fiber-optic sensor uses a 100 W quartz halogen light as the source, and a rotating filter wheel selects between green and red light to illuminate the sample under study. Light passes down the fiber-optic input fiber and is scattered from the polystyrene light-scattering microspheres so that adequate light is collected and sent back to the receiving fiber (Peterson and Vurek, 1984).

The green light returning to the sensor varies as a function of the pH, whereas the red light does not vary with pH. Because the red light is generated by the same source as the green light and travels the same optical path to the detector, any changes in the optical system are reflected in changes in the red light received by the detector. Thus, when the intensity of the green light received by the detector is divided by the intensity of the red light received, any changes in the optical system are compensated for by this ratiometric method.

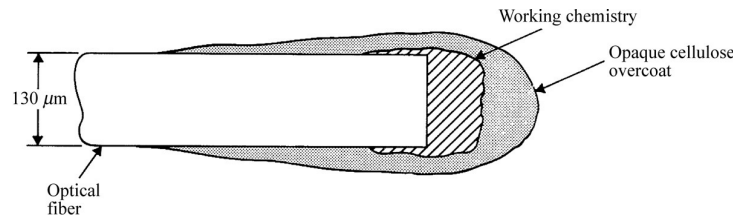


Figure 10.12 A single-fiber intravascular blood-gas sensor excites fluorescent dye at one wavelength and detects emission at a different wavelength. The following modifications are made to the sensor tip: pH: Chemistry—pH-sensitive dye bound to hydrophilic matrix. P_{CO_2}: Chemistry—Bicarbonate buffer containing pH-sensitive dye with silicone. P_{O_2}: Chemistry—Oxygen-sensitive dye in silicone. (From J. L. Gehrich, D. W. Lübbers, N. Optiz, D. R. Hansmann, W. E. Miller, J. K. Tusa, and M. Yafuso, "Optical fluorescence and its application to an intravascular blood gas monitoring system,"*IEEE Trans. Biomed. Eng.*, 1986, BME-33, 117–132. Used by permission.)

intravascular blood-gas probe for pH (Gehrich *et al.*, 1986). The pH-sensitivity range is approximately equal to $pK_a \pm 1$.

Figure 10.12 is a diagram of the intravascular blood-gas sensor, in which chemistries are covalently bonded through a cellulose matrix attached to the fiber tip. An opaque cellulose overcoat formed over the matrix provides mechanical integrity and optical isolation from the environment.

The underlying principle of fluorescent measurement is that fluorescent dyes emit light energy at a wavelength different from that of the excitation wavelength, which they absorb. This can be seen in Figure 10.13, which gives the fluorescence spectra of a pH-sensitive dye. The excitation peak wavelength for the acidic form of the dye is 410 nm, whereas the excitation peak wavelength for the basic form of the dye is 460 nm. It is also apparent that the emission spectra for both the acidic and the basic forms of the dye have a peak at 520 nm. Because of the separation between the excitation and emission wavelengths, it is possible to use a single optical fiber both for the delivery of light energy to the sensor and for its reception from that sensor.

Intravascular dye fluorescence sensors must be stable enough to maintain accuracy for up to three days of use within the patient. Cost and shelf life of this disposable product must also be considered. In addition, the dye must be able to follow physiological changes in the blood-gas parameters and thus must have sufficient dynamic range and time response (Gehrich *et al.*, 1986).

The ratiometric principle, or two-wavelength approach, is used to design an optical measurement system that is independent of system and other parameters, which include (1) loss of the optical signal as a result of fiber bending, (2) optical misalignment, and (3) other changes in the optical path that could be incorrectly interpreted as changes in the concentration of the analyte being measured. The ratiometric approach is undertaken by selecting fluorescent dyes with two absorption or emission peaks or by providing a

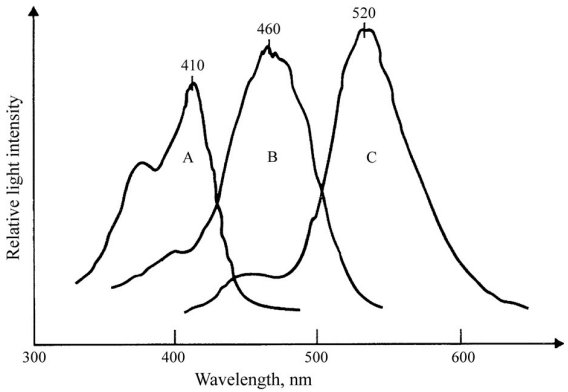


Figure 10.13 This pH-sensitive dye is excited at 410 and 460 nm and fluoresces at 520 nm: (A) the excitation spectrum of the acidic form of the dye, (B) the excitation spectrum of the basic form of the dye, and (C) the emission spectrum of the acidic and basic forms of the dye. (From J. L. Gehrich, D. W. Lübbers, N. Optiz, D. R. Hansmann, W. E. Miller, J. K. Tusa, and M. Yafuso, "Optical fluorescence and its application to an intravascular blood gas monitoring system,"- *IEEE Trans. Biomed. Eng.*, 1986, BME-33, 117–132. Used by permission.)

mixture of dyes at the sensor tip, one that is sensitive to the measured parameter and one that is not (reference wavelength, which is affected only by the optical system parameters). In the foregoing example, the emission due to excitation at 410 nm represents the relative amount of the basic phase, and the emission due to excitation at 460 nm represents the relative amount of the acidic phase. The ratio of these phases represents the pH.

FLUORESCENCE OPTICAL P_{CO_2} SENSOR

The P_{CO_2} sensor uses the same pH-sensitive fluorescent dye as the pH sensor described before. The operation of this sensor is similar to that of the electrochemical Severinghaus P_{CO_2} electrode described in the previous section in that a pH-type sensor is used as the basic sensing element to detect P_{CO_2}. Carbon dioxide comes to equilibrium with a mixture of a pH indicator in bicarbonate buffer. There is a direct relationship, based on the Henderson–Hasselbach equation, between the pH change in a bicarbonate solution and the CO_2 concentration in that solution. Thus a change in pH in an isolated bicarbonate buffer with a changing P_{CO_2} is measured. This buffer is encapsulated by a hydrophobic gas-permeable silicone matrix that provides ionic isolation and mechanical stability for the measurement system.

As before, an optical cellulose overcoat ensures optical isolation of the sensor chemistry from the environment. CO_2 equilibrates rapidly across the silicone membrane and causes a change in the pH. The concentration of the bicarbonate buffer is selected such that a sufficient pH change is detectable with appropriate accuracy and sensitivity over the physiological range for CO_2,

FLUORESCENCE OPTICAL P_{O_2} SENSOR

One approach for a fiber-optic P_{O_2}, or oxygen partial pressure, sensor makes use of the principle of fluorescence or luminescence quenching of oxygen. In this quenching process, energy is absorbed and lost by various processes, such as vibration of the molecule (heat) and emission of the light as fluorescence or phosphofluorescence. With oxygen present, these molecules provide collision paths and transfer of energy to the oxygen molecule, which competes with the energy decay modes, and luminescence is decreased by the increasing loss of energy to oxygen.

Figure 10.14 shows the fluorescent spectra of oxygen-sensitive dye for both the excitation and the emission.

The P_{O_2} probe is similar in design to the pH sensor. The principle of its operation is that when these fluorescent quenching dyes are irradiated by light at an appropriate wavelength, they fluoresce in a nonoxygen atmosphere for a given period of time. However, when oxygen is present the fluorescence is quenched—that is, the dye fluoresces for a shorter period of time. The period of dye fluorescence is inversely proportional to the partial pressure of oxygen in the environment. This leads to a poor signal-to-noise ratio at high P_{O_2} values, because the high O_2 levels quench the luminescence, which results in a small signal at the detector. In Figure 10.15, fibers and inert beads are enclosed in an oxygen-permeable hydrophobic sheet such as porous polypropylene.

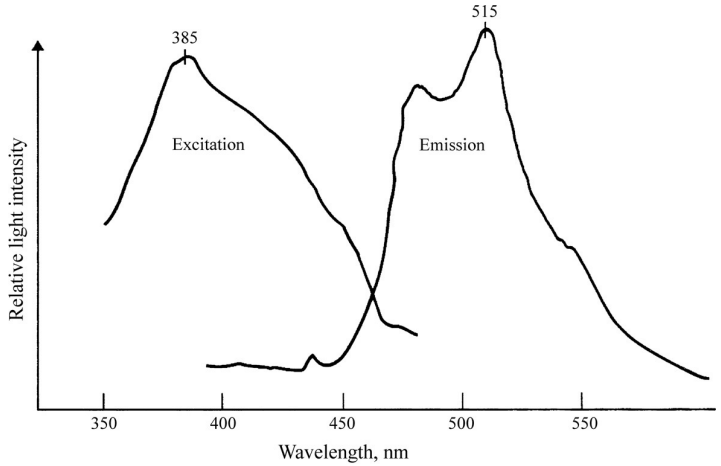


Figure 10.14 The emission spectrum of oxygen-sensitive dye can be separated from the excitation spectrum by a filter. (From J. L. Gehrich, D. W. Lübbers, N. Opitz, D. R. Hansmann, W. W. Miller, J. K. Tusa, and M. Yafuso, "Optical fluorescence and its application to an intravascular blood gas monitoring system," *IEEE Trans. Biomed. Eng.*, 1986, BME-33. 117–132. Used by permission.)

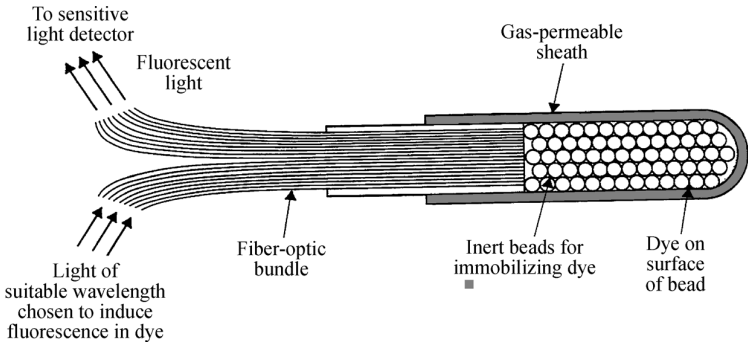


Figure 10.15 In a fiber-optic oxygen sensor, irradiation of dyes causes fluorescence that decreases with P_{O_2}. [From R. Kocache "Oxygen analyzers." in J. G. Webster (ed.), Encyclopedia of Medical Devices and Instrumentation. New York: Wiley, 1988, pp. 2154–2161. Used by permission.]

The P_{O_2}-measurement instrument includes both optical and electronic systems. The instrumentation system as designed uses plastic optical fibers because of their mechanical strength and flexibility; they allow for a sharp bending radius. The light returning from the sensor passes through a dichroic filter, which separates the green fluorescent light from the blue excitation light, and the latter is scattered by the probe back into the return fiber. Photomultiplier tubes are used in this application to convert the light signal into a current, and then a current-to-voltage converter is used to provide the voltage proportional to the blue and the green light. The blue/green ratio is taken, and the P_{O_2} output is calculated according to the Stern–Volmer equation.

There is a range of quenching-base sensors. They include sensors based on transition metal quenching of ligand fluorescence and on iodine quenching of rubrene fluorescence (Seitz, 1984).