Blood Chemistry Measurement

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78.1 Introduction

The study of blood and its effects on sustaining life date back to the start of understanding human anatomy. There is a diverse background associated with blood chemistry, and knowledge of the makeup and purpose of blood and its components is growing by leaps and bounds. The typical discussion and history of blood chemistry relate to oxygen transport and its effects. This chapter will deal mainly with the measurement of oxygen related to health and critical-care applications, with limited descriptions of noncritical health status measurements. The beginning of blood analysis and the measurement of a person's health status were not practically and conveniently performed until technology advanced. It has been within the last 40 years that routine monitoring began in critical care. Critical-care health monitoring is the focus of this discussion because it has been one of the main motivations for developing analytical measurements. However, most critical-care monitoring has led to diagnostic tools and functional assessment of blood and its components.

Current health care professionals use a variety of common analytical measurements to assess and maintain a person's health. The measurements of interest include hemoglobin, hematocrit, blood gases (O_2 and CO_2), pH, glucose, and concentrations of electrolytes. Blood gas analysis is divided into a wide variety of specific tests. The tests include arterial, mixed venus, and **transcutaneous** oxygen tension; carbon dioxide tension; oxygen and carbon dioxide concentration; oxygen saturation. Each test has a specific purpose in diagnostics. Continuous real-time monitoring of oxygen saturation by pulse oximetry measurements is routinely used in anesthesia and critical-care settings. However, to determine heart and lung efficiency or true tissue oxygenation, other measurements are required. Different measurements are required because of the complex nature of the human body, and no one measurement gives a complete picture of overall status. For example, arterial oxygen saturation does not give a true cardiac output efficiency nor does it give a true tissue oxygenation state. Measurements can also be very misleading. During normal conditions, the measured values correspond very well. For example, under a given temperature and pH, oxygen saturation corresponds to oxygen tension. A fixed relationship occurs under

these conditions. This does not mean the same relationship applies to all circumstances. A change in temperature or pH will shift the oxygen tension to a saturation relationship.

78.2 Background

Most blood chemistry measurements have a long history. In the late 1800s the hydrogen (H^+) electrode was used to measure pH and, in 1925, John Peters at Yale showed a relationship between pH and CO_2 content. The measurement of blood gas started in the early 1920s with *in vitro* analysis of oxygen saturation. It was not until much later, however, that common patient care measurements were accepted. For example, it was not until 1952 in Copenhagen that pH monitoring was performed on artificially ventilated patients. The monitoring of pH was the start of the clinical use of blood gas monitoring. Until that time, physiological measurements were performed only in the laboratory setting. Electrochemical measurements were more common, and performed with a Clark electrode or similar electrode arrangement. Leland Clark made a working model of an oxygen electrode around 1950, which introduced a practical method of measuring oxygen tension. The probe was originally developed to aid in heart bypass instruments. Not long after the Clark electrode was developed, optical transmission analysis tools were developed. The measurement of oxygen saturation, called oximetry, was one of the early optical measurements. Oximetry has a long history dating back more than 50 years to the 1930s. Matthes and Millikan recorded the earliest noninvasive reading around 1935, and Glen Millikan introduced the name **oximetry** in 1942. A real research effort was started during World War II as a part of military aviation. It took almost 60 years for pulse oximetry to become a standard piece of equipment in operating rooms, critical-care units, and emergency health care. On January 1, 1990, the American Society of Anesthesiologist (ASA) made intraoperative monitoring with pulse oximetry a standard [1]. Despite the acceptance of pulse oximetry and its benefits, there still has been little change in pulse oximetry techniques. A good historic perspective on most blood gas analysis is written in a series of articles in the *Journal of Clinical Monitoring* and in *International Anesthesiology Clinics* [2-7].

Oxygen Delivery

As Severinghaus and Astrup [6] note in the history of blood gas analysis, nothing is more important than oxygen supply for life. Therefore, it can be argued that oxygen content and consumption should be one of the most important things to monitor in critical and unconscious patients. It is important to look at the mechanisms for oxygen transport. All human tissue needs oxygen to function, and it is therefore critical to understand how oxygen is delivered. **Hemoglobin** is the binding agent for oxygen in blood, but it is necessary to oxygenate the blood and move it to the tissue that uses the oxygen. A simple explanation is as follows. Oxygen is diffused into blood via the lungs. Blood is then circulated by a system of **arteries**, **veins**, and **capillaries** where the oxygen is diffused to tissue. The tissue also diffuses carbon dioxide to blood in the capillaries, which transports back to the lungs, where the carbon dioxide is diffused to the lungs for expiration. Figure 78.1 shows a diagram of the general makeup of the components of interest. This is a very simplistic view of blood and oxygen transport. There are many other regulatory factors, such as sodium, potassium, calcium, pH, glucose, and so forth, but this simplistic view is a good starting point to understand the measurements that follow. It is also important to note that several measurement options and locations exist for gathering the same or similar data. This makes understanding the interaction and importance of different measurements difficult. For example, arterial and venus blood oxygen tensions are interdependent, but have different readings. Different physical states and health problems cause varying interdependence. A simple requirement to identify whether or not cell tissue is being properly oxygenated becomes very complex. Oxygen delivery to tissue is determined by the differential in partial pressure between cell tissue and capillaries. The partial pressure difference determines the rate and efficiency of diffusion. However, the rate is also dependent on blood flow, concentration of hemoglobin, and the saturation of oxygen. No individual parameter or measurement gives a complete picture of cell oxygen consumption.

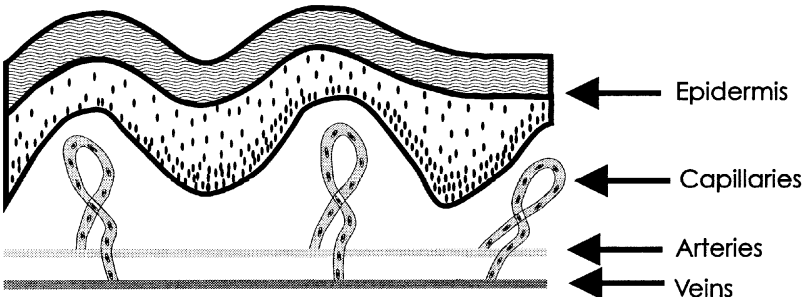

FIGURE 78.1 Components involved in oxygen transport. Oxygen is delivered to the epidermis by arteries and exchanged through the capillaries. Veins return the by-product.

Blood Hemoglobin

Blood contains many components, but hemoglobin is the main transporter of oxygen (O_2) to tissue. In addition, blood is responsible for removing the by-product of oxygen expenditure, carbon dioxide (CO_2). Hemoglobin is a good binding agent for oxygen, producing oxyhemoglobin (O_2Hb), also called oxygenated hemoglobin. Hemoglobin is a combination of four peptide chains, each containing several hundred amino acids. Hemoglobin is composed of two elements, heme and globin. Heme is the iron pigment of the electrolytes, and globin is a simple protein. Hemoglobin contains about 6% heme and 94% globin. Hemoglobin is the principal component of red blood cells. Unbound hemoglobin is called deoxyhemoglobin (RHb), or reduced hemoglobin. Hemoglobin also binds to produce additional components, such as, carboxyhemoglobin (COHb), and methemoglobin (MetHb). Methemoglobin is a result of oxidation of ferrous iron in hemoglobin [8]. There are other compounds that are found in blood, but they are usually in very low concentrations and are of interest in more specialized cases.

Measurement Methods

Most blood chemistry analysis measurements are derived from one of two methods: electrochemical and optical. There are other specific measurement techniques, such as gas chromatography, but they are more of a specialized measurement and will not be addressed. Electrochemical measurements are based on the Clark electrode with the blood component or electrolyte of interest using a different ion-specific electrode. The Clark electrode consists of an electrode in a medium. The first electrode for oxygen tension measurements was a platinum electrode and a silver anode. The current or voltage generated at the electrode is measured and is proportional to the amount of that component. The relationship is usually a calculated concentration derived from empirical data. For optical measurements, there are many approaches, but all are based on absorption, reflection, scattering, and fluorescence techniques. One optical probe has been called an optode. The optode is a cross measurement of optical fluorescence and electrochemical measurement. Figure 78.2 shows a diagram of one configuration for an optode arrangement. In an optode, an ion-selective membrane is used to diffuse an ion or compound into the fluorescent dye measurement chamber. The ion is associated with a fluorescent dye, excited with a light source, and the emitted fluorescent light is measured. Many measurements can be obtained, such as oxygen tension, carbon dioxide tension, pH, potassium concentration, calcium concentration, and chloride concentration.

Measurements are listed in one of four categories, (1) invasive or (2) noninvasive measurements and (3) continuous or (4) periodic measurements. **Invasive** measurements can be continuous or periodic, and can be performed *in vivo* or *in vitro*. Invasive **catheter** sensors can record real-time data continuously with no loss of blood sample. **Intra-arterial** measurements can be drawn periodically for minimally invasive measurements with no sample loss, or samples can be withdrawn and discarded. Periodic samples can also be drawn for analysis and measured in a physically different location at a later time. Noninvasive measurements can also be continuous or periodic, but are generally on patient, real-time measurements.

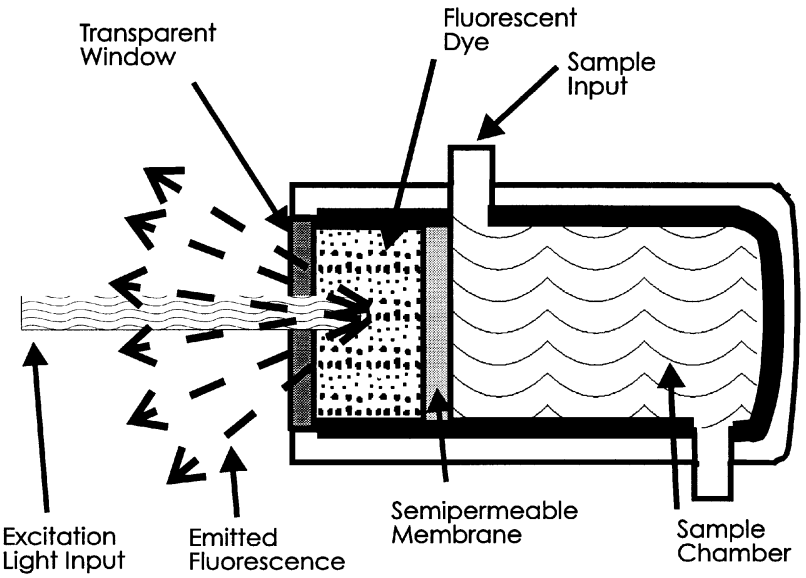

FIGURE 78.2 Chemical fluorescent optode for oxygen tension measurements. Light input through a window excites encased dye. The sample interacts with the dye through a semipermeable membrane. The sample is introduced to the dye via a flow-through chamber.

Noninvasive measurements are always preferred over invasive measurements, because invasive measurements increase risk of infection and usually mean some delay between the time of acquisition and the time results are available. However, noninvasive measurements are not always as accurate as invasive measurements.

78.3 Measurements and Techniques

There are typically several measurement techniques that yield the same or similar data. Each technique will give information about different physical states, such as cardiac efficiency or tissue oxygenation state. For example, oxygen saturation and partial pressure of oxygen are related. However, the exact relationship is dependent on pH and temperature. A text by Kenneth McClatchey on *Clinical Laboratory Medicine*, the International Federation of Clinical Chemistry (IFCC), and instrument operator's manuals are good references for laboratory protocols relating to invasively drawn samples [9,10]. Sample preparation and handling protocols, as well as standard measurement levels and physical states relating to excess or deficient readings are detailed. The range, price, and suppliers for instrumentation are extensive. A standard midrange laboratory blood gas analyzer can cost from $5,000 to $50,000. The more common measurements are described below.

Hemoglobin and Hematocrit Concentration

The total concentration of hemoglobin (CtHb) or **hematocrit** (Hct) indicates the oxygen-carrying potential of blood. The combination of amount of hemoglobin, partial pressure of oxygen or percent oxygen saturation, and rate of flow of hemoglobin determines the amount and efficiency of tissue oxygenation. Invasively drawn samples are typically used to measure hemoglobin and hematocrit values.

Hemoglobin

The total hemoglobin concentration is not the same as the red blood cell count, because red blood cells have different amounts of hemoglobin. The total hemoglobin concentration is measured optically by

absorptive intensity at the isosbestic point. The Beers–Lambert law, also referred to as Beer's law, shown in Equation 78.1 regulates the absorptive property of a substance.

$$I_t = I_0\, e^{-DC\alpha_e} \tag{78.1}$$

I_t is the transmitted intensity, I_0 is the incident intensity on the sample, D is the distance light travels through the substance, C is the concentration of the solution, and α_e is the extinction coefficient at a specified wavelength. The isosbestic point is the crossover point in extinction curves for oxyhemoglobin and reduced hemoglobin. The wavelength of 805 nm is an isosbestic point, and at this wavelength the absorption is independent of hemoglobin type. If blood is assumed to be composed of only O_2Hb and RHb, then the absorbance at the isosbestic point determines the total concentration of Hb. The most common error in assuming only O_2Hb and RHb is during elevated COHb. COHb, however, generally is optically indistinguishable from O_2Hb for absorption. An alternative approach to isosbestic measurement is to calculate CtHb. Assuming only O_2Hb and RHb, the total Hb concentration is simply the sum of the two concentrations. A more accurate sum is shown in Equation 78.2.

$$\text{CtHb} = \text{O}_2\text{Hb} + \text{RHb} + \text{COHb} + \text{MetHb} \tag{78.2}$$

Here the four most common Hb derivatives are used to calculate a more accurate CtHb. The individual Hb derivatives are measured as outlined below.

Hematocrit

Hematocrit is the volumetric fraction occupied by red blood cells and is generally measured by conductivity. Hematocrit is also referred to as the packed cell volume (PVC). Hematocrit can be determined by conductivity based on the plasma ion content. Hematocrit does not contribute to the conductivity and therefore is inversely proportional to the conductivity. Hematocrit can also be determined optically in various ways. One way is through optical density measurements, where the total optical density is the sum of optical absorbance and optical scattering density. The total optical density is linearly proportional to Hct, and, at clinically relevant Hct levels of 20 to 40%, scattering is dominant over absorption. One optimization study showed that an optimum wavelength of 624 nm, at a measured angle of 90° from the incident light, gave an inverse linear intensity to the Hct level [11].

Oxygen Tension

Oxygen tension or the partial pressure of oxygen (PO_2) is a common measure of oxygenation states. The partial pressure of oxygen in hemoglobin determines how well oxygen is delivered to the cell tissue of the body. The more common partial pressure reference is arterial partial pressure of oxygen (PaO_2). If the partial pressure of oxygen is higher than the surrounding tissue, oxygen is diffused to the tissue. If the partial pressure is lower than the tissue partial pressure, no oxygen is diffused to the tissue, and tissue damage can start to occur. Equation 78.3 shows Henry's law.

$$C = \alpha_s * \text{PO}_2 \tag{78.3}$$

C is the concentration of oxygen (O_2), α_s is the solubility coefficient, and PO_2 is the partial pressure of oxygen. In diffusion, a partial pressure between the tissue and blood supply is trying to maintain equilibrium. Diffusion will occur until the two partial pressures are equal. Equation 78.4 shows diffusion equilibrium.

$$\text{PO}_2 = C_1 / \alpha_{s1} = C_2 / \alpha_{s2} \tag{78.4}$$

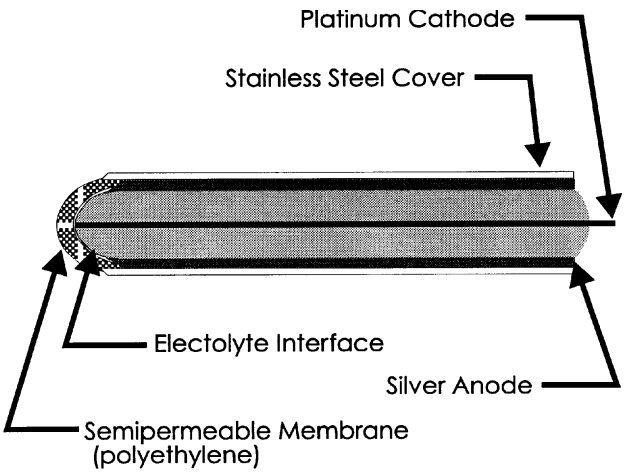

FIGURE 78.3 Clark-type needle electrode for PO_2 measurements. Oxygen ion interaction through the membrane tip allows the ion concentration to be measured by dissimilar metals.

The rate of oxygen diffusion is dependent on the difference in partial pressure. The larger the difference in partial pressure, the faster the diffusion rate.

The partial pressure difference is partially determined by the oxygen delivery (D_O) rate. Oxygen delivery can be identified as the concentration of arterial oxygen minus the concentration of venus oxygen, times the rate of blood flow (R), as shown in Equation 78.5.

$$D_O = \left(C_aO_2 - C_vO_2\right) * R \tag{78.5}$$

C_aO_2 is the arterial oxygen concentration, and C_vO_2 is the venus oxygen concentration. The oxygen delivered, however, is not the oxygen delivered to the tissue. Some oxygen is transpired through the skin. The transpired oxygen allows an alternative approximation measurement of arterial oxygen tension. The more common oxygen tension measurement is arterial oxygen tension. Oxygen tension can be measured electrochemically, transcutaneously, or optically. The techniques are listed below.

Electrochemical PO_2 Measurements

Electrochemical measurements of PO_2 are obtained using a basic Clark electrode with a platinum electrode and a silver/silver chloride reference electrode. Figure 78.3 shows a diagram of one type of Clark electrode. The electrode provides a path for the reduction in Equation 78.6.

$$O_2 + 4e^- \rightarrow 2O^- \tag{78.6}$$

The platinum electrode has an affinity with oxygen. If a reference PO_2 at the electrode is known, preferably zero, then the current depends only on the oxygen tension variations of the sample. Early electrodes were consumption measurements where the oxygen is removed and the sample is altered. Oxygen was attracted to the electrode and a current proportional to the oxygen content was observed. Improved electrodes use a semipermeable, constant diffusion membrane. An electrolyte is used with the membrane to improve response, longevity, and stability. An exposed electrode will become coated in whole blood and the sensitivity will degrade. The membrane and electrolyte allow the diffusion of oxygen without the sample directly contacting the electrode. In addition, a properly designed membrane reduces the stirring effect noted by Clark and others using an exposed electrode. The size and location of electrodes have been extensively studied. The exact setup for the electrode can be varied for optimum performance in different

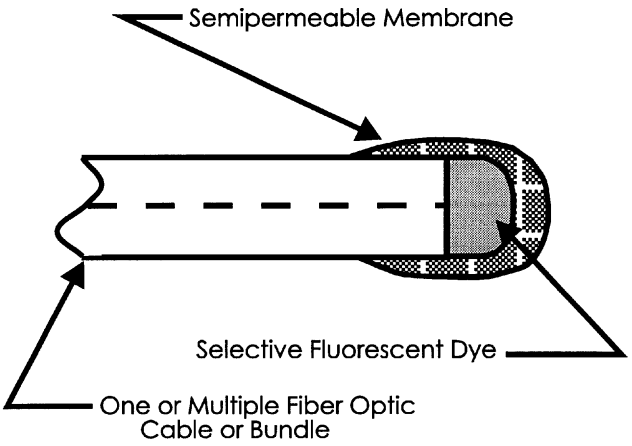

FIGURE 78.4 Chemical fluorescent fiber-optic probe. Dye is encased on a fiber tip by a membrane. Light enters the dye through the fiber and the emitted fluorescence is returned via the fiber. The amount of fluorescence is an indication of the interaction between the dye and the substance in which the fiber tip is placed.

areas. For example, a less permeable diffusion layer reduces the stirring effect and makes the probe more stable, but the result is a slower response time [3,6,12,13].

Transcutaneous Partial Pressure of Oxygen

Partial pressure and oxygen saturation are not always true indicators of actual tissue oxygen consumption. It has been known since 1851 that oxygen is respired from living tissue. A combination of heat and optical means can be used to determine the amount of oxygen expired and correlated to arterial partial pressure under controlled conditions. This method of measuring arterial oxygen partial pressure is called transcutaneous oxygen partial pressure ($P_{tc}O_2$). There are, however, many complications in the measurement of $P_{tc}O_2$. Many studies have tried to correlate arterial oxygen saturation with the measured expired oxygen. The most accurate measurements are made by maintaining a constant temperature as high as 45°C, to assure perfusion. This causes the complication of burns to skin, and sensors need to be moved on a regular basis.

Measurements can be performed polargraphically using Clark electrodes or alternatively by mass spectroscopy. Because of the nature of $P_{tc}O_2$, adult measurements are not common, but premature infant hemoglobin and skin are more responsive to $P_{tc}O_2$ measurements, which have had a place in monitoring neonatal oxygenation [7,14].

Optical-Based PO_2

The are many variations on optical-based blood gas measurements. The basic optical measurement for PO_2 is a **fluorescent** measurement. A fiber-optic cable is used to excite a fluorescent dye remotely. The excited dye emits a higher-wavelength signal, known as Stokes shift. The emitted signal is measured and is correlated to the PO_2 value. Figure 78.4 shows a configuration for a fluorescent sensor. Oxygen is used as a fluorescence quencher. Oxygen has a fluorescence-quenching property and attenuates the fluorescent intensity. A zero state can be measured by filling the sample chamber with a zero oxygen concentration mixture. The measured intensity is compared with the incident light source and is used to calculate the concentration of the sample. For oxygen tension, the measurement is done by a fluorescent electrochromic dye, such as pyrenebutyric acid, with an ion-selective membrane such as silicone rubber. The oxygen tension is than calculated using the Stern–Volmer quenching formula, shown in Equation 78.7, and the PO_2 value is calculated empirically.

$$I_0 / I = 1 + K * \mathrm{PO}_2 \quad \text{or} \quad I\left(\mathrm{PO}_2\right) = I_0 / \left(1 + K * \mathrm{PO}_2\right) \tag{78.7}$$

$I(PO_2)$ and I_0 are the relative fluorescence intensities in the presence of oxygen and in the absence of oxygen, respectively. K is the overall quenching constant and is given in Equation 78.8.

$$K = k^+ * \alpha_O * \tau_0 \tag{78.8}$$

k^+ is the collisional quenching constant, α_O is the oxygen solubility coefficient, and τ_0 is the mean lifetime of the excited state at zero PO_2. The ratio of I to I_0 is then plotted against the PO_2 value. This plot is used to calibrate the sensor empirically for different PO_2 values. $1/I_0$ is the intercept, and the slope is K/I_0. The system is empirically derived and errors can occur with varying excitation intensity. One way to correct for intensity variations is to use a reference fluorescence dye that is not affected by oxygen quenching. The same procedure can be used to calculate the percent oxygen concentration [15,16].

Oxygen Saturation

There are several readings that are interrelated regarding oxygen saturation. The most common measurements are arterial blood saturation (S_aO_2), mixed venus blood saturation (S_vO_2), and **photoplethysmogram** arterial blood saturation (S_pO_2). S_pO_2 measurements, also called pulse oximetry measurements, are arterial measurements, but are only related to true arterial saturation. Care must be taken in knowing which measurements are actually taken, because of inaccuracies in various measurements. Saturation measurements can be performed invasively using reflectance or fluorescence oximetry, or noninvasively by transmission or reflectance photoplethysmogram readings.

There is an important relationship between oxygen saturation and partial pressure. A plot shown in Figure 78.5 of arterial oxygen saturation vs. arterial partial pressure is called the oxygen hemoglobin

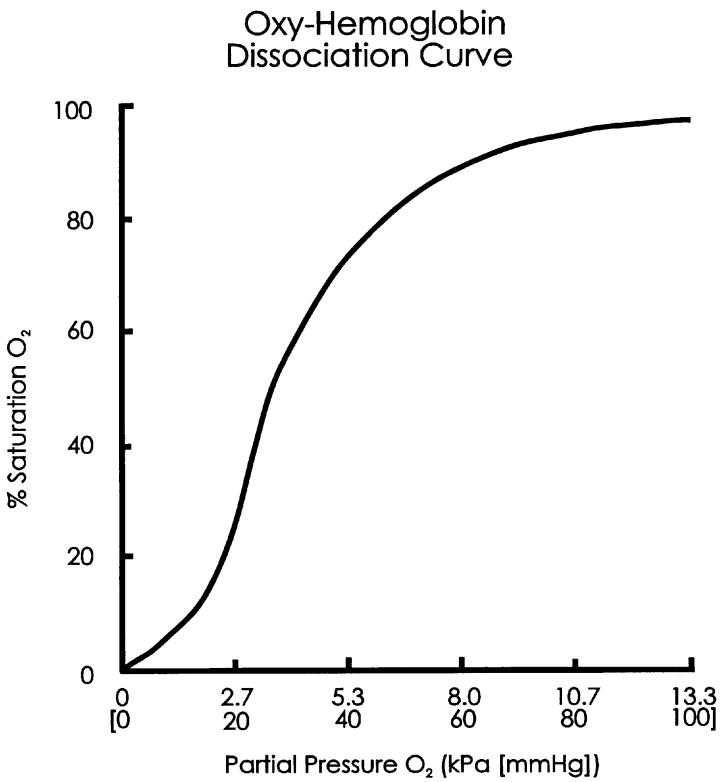

FIGURE 78.5 Oxygen saturation vs. PO_2 oxygen hemoglobin dissociation curve. The partial pressure shows a sigmoidal relationship to the percent oxygen saturation.

dissociation curve. Partial pressure falls at a linear rate and is a good indicator of changing oxygen delivery to cell tissue. The percent oxygen saturation is nonlinear with respect to partial pressure in a sigmoidal relationship. A partial pressure of 13.3 kPa (100 mmHg) is effectively 100% saturation. An increase in partial pressure above 13.3 kPa (100 mmHg) indicates free oxygen not bound to hemoglobin and in that state does not contribute much to tissue oxygenation. This means the percent saturation gives a delayed notice of desaturation. In a patient with healthy lungs, partial pressure values of greater than 24.0 kPa (180 mmHg) can be measured. A 50% decrease in partial pressure could go undetected using the percent oxygen saturation. The oxygen dissociation curve, as mentioned previously, is dependent on pH and temperature. Under normal conditions, the relationship between partial pressure and saturation is maintained, but under altered conditions, such as low perfusion, the relationship can deviate.

Roughton and Severinghaus [18] describe a computation to approximate the dissociation curve based on the Hill equation. The Hill equation is given in Equation 78.9.

$$Y = \left[\left(PO_2 / P_{50}\right)^n\right] \Big/ \left[1 + \left(PO_2 / P_{50}\right)^n\right] \tag{78.9}$$

Y is the oxygen saturation, PO_2 is the oxygen partial pressure, and P_{50} is the partial pressure at 6.7 kPa (50 mmHg). The P_{50} point is determined by the temperature and pH of the sample. Temperature and pH correction equations are also presented in References 5, 7, and 17 through 19.

Transmission Oximetry

Continuous S_aO_2 and S_vO_2 measurements are used to help evaluate whether oxygen is being adequately delivered to tissue. Oxygen is either consumed or expired. If oxygen is delivered to tissue and not consumed, then tissue oxygenation is not adequate. Early oximeters used invasively drawn samples and light transmission to measure oxygenation. The basic concept in oximetry is to transmit light through a blood sample, and blood absorbs a determined amount of light according to Beer's law, shown in Equation 78.1. For oximetry applications, hemoglobin is assumed to be composed of only two substances, oxygenated hemoglobin or oxyhemoglobin (O_2Hb) and deoxygenated or reduced hemoglobin (RHb). This is a very simplistic approach, but it is the basis for most oximetry measurements. For *in vivo* measurements, the path length for the light is constant and known. Both the O_2Hb and RHb can be measured simultaneously by using two separate wavelengths. Flash lamps and filter wheels were originally used to illuminate blood samples, and the transmitted signals were measured. Current technology uses light emitting diodes (LEDs) and alternate on and off cycles.

If two light wavelengths are used and the two substances have different extinction coefficients (α_e), or equivalently attenuation coefficients (σ), then the percentage of each substance can be calculated. The extinction coefficients of hemoglobin are well documented. Figure 78.6 is a plot of the extinction coefficients for oxyhemoglobin, reduced hemoglobin, carboxyhemoglobin, and methemoglobin. The two wavelengths of light yielding good results for oximetry are the red (660 nm) and infrared (940 nm) wavelengths. Red has the largest difference between the two extinction curves, and infrared has maximal difference after the isosbestic point where the two extinction curves cross. The functional arterial oxygen saturation (S_aO_2) is calculated using Equation 78.10 given the concentration of O_2Hb and RHb.

$$\text{Functional } S_a O_2 = C_o \Big/ \left(C_o + C_r\right) * 100 \tag{78.10}$$

The concentration of oxyhemoglobin is given by C_o and the concentration of deoxygenated hemoglobin is given by C_r. This formula must be adjusted for other contents in blood which influence measurements if present, such as carboxyhemoglobin and methemoglobin. The more accurate fractional saturation is given as the total hemoglobin concentration (tHb) in Equation 78.11.

$$\text{Fractional } S_a O_2 = C_o \Big/ \left(\text{tHb}\right) * 100 = C_o \Big/ \left(C_o + C_r + C_c + C_m\right) * 100 \tag{78.11}$$

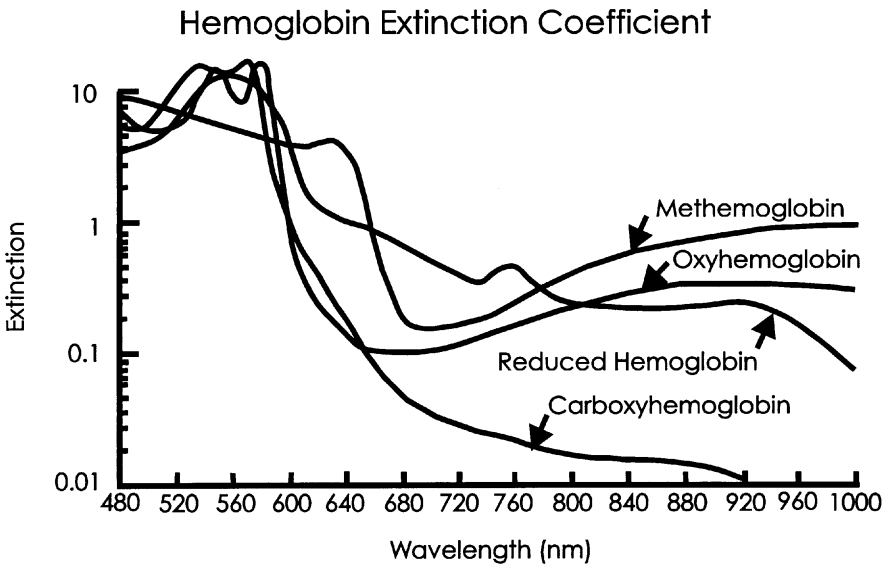

FIGURE 78.6 Hemoglobin extinction coefficients plotted by wavelength. The light extinction coefficient varies by wavelength and type of hemoglobin.

C_c is the carboxyhemoglobin (COHb) saturation and C_m is the methemoglobin (MetHb) concentration. To account for COHb and MetHb accurately, four transmission wavelengths would be required, or for accurate S_aO_2 readings the total Hb concentration needs to be known or measured [5,7,20].

Pulse Oximetry

Pulse oximetry is based on the transmission, absorption, and dispersion of light as it passes through hemoglobin. Beer's law, as stated in Equation 78.1, determines the transmission of light through a substance. For pulse oximetry, the light illuminates both arterial and venus blood and the light must traverse all tissue between light source and receiver. Figure 78.7 represents the light path, and indicates a variable (AC) path length as well as a constant (DC) path length.

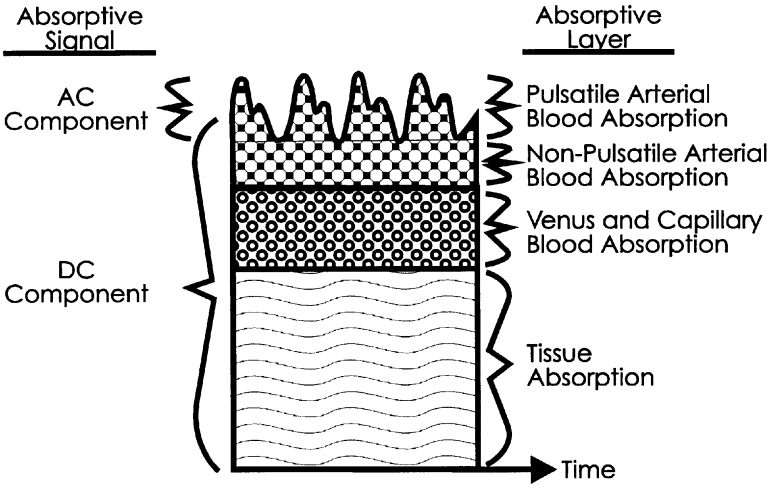

FIGURE 78.7 Absorption components encountered during transmission oximetry. The arteries, veins, capillaries, and tissue absorb light. The total light absorbed has a steady state (DC) and a varying (AC) signal component. The varying signal is due to the pulsatile arterial volume change.

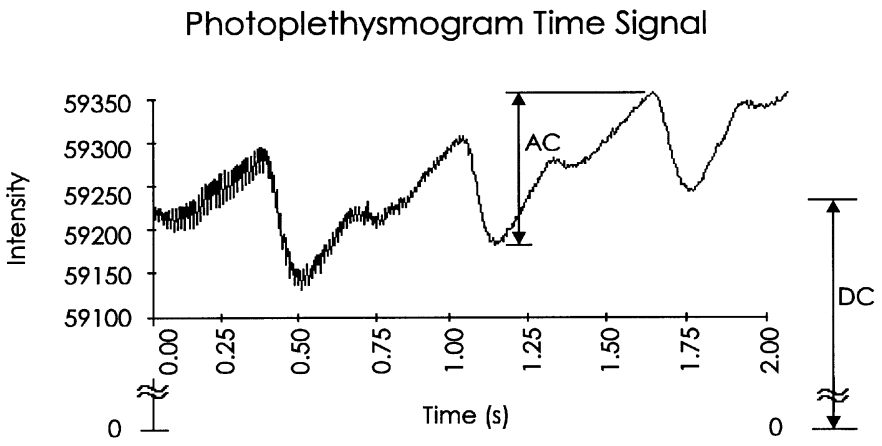

FIGURE 78.8 Photoplethysmogram with AC and DC components labeled. The light intensity transmitted through the finger varies with time. The signal contains a constant (DC) component and a varying signal (AC) component. The varying signal is due to the pulsatile arterial volume change.

To calculate the pulse oximetry digital photoplethysmogram (DPP) oxygen saturation (S_pO_2), two equations are used. An example of a DPP signal is shown in Figure 78.8 and it indicates the AC and DC DPP components. The first step is to use the red and infrared time signal to calculate an *R* value. The *R* value is the normalized ratio of the red to infrared transmitted light intensity and is shown in Equation 78.12.

$$R = \left(AC_r / DC_r\right) \Big/ \left(AC_i / DC_i\right) \tag{78.12}$$

The *R* value for two specific light wavelengths can be plotted against a measured S_aO_2 value, as shown in Figure 78.9. A linear approximation can then be used to calculate a S_pO_2 value. The empirical linear approximation for Figure 78.9 is listed in Equation 78.13.

$$S_pO_2 = 110 - 25R \tag{78.13}$$

The empirical approximation is used to correct for errors in the measured values. Pulse oximeters currently on the market use weighted moving average (WMA) techniques to identify the transmitted AC and DC DPP components. The DC component is the averaged signal intensity. The AC signal is computed using the WMA as a bandpass filter to single out only the AC cardiac signal.

There are many areas where pulse oximetry has limitations. One limitation was already mentioned, and that is the assumption of only two substances in hemoglobin. For most measurements, the percentage of these substances is small enough not to affect the pulse oximetry measurements. There are cases in emergency care when these substances may be present and do affect readings. At present, the user must be aware of the limitations and not use the pulse oximetry reading if the person has any of the additional substances. The solution to this problem is to use additional light sources to calculate the additional substance. The first pulse oximeters tested used a multiple-wavelength light source and could easily be implemented in new instruments.

A second limitation of pulse oximetry is background light. Because pulse oximetry currently uses transmitted light, the photodiode receiver is susceptible to ambient light. Ambient light can be from indoor lighting, sunlight, or phototherapy lights. To correct for this problem, a third light measurement can be collected with no light source, and subtracted from the transmitted intensity. Using a digital microprocessor, the subtraction can be easily performed.

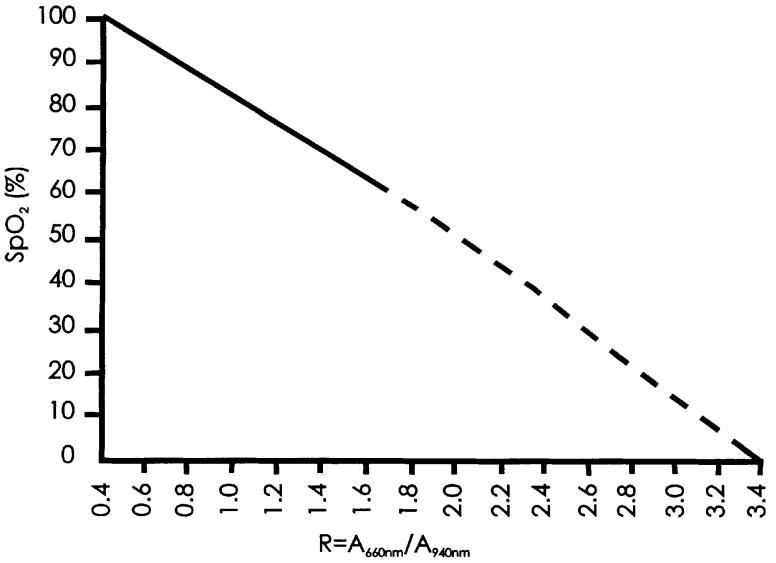

FIGURE 78.9 Measured oxygen saturation vs. *R* value. Empirically derived 660 to 940 nm ratio and oxygen saturation. The values are used to formulate a first-order equation to calculate the pulse oximetry saturation.

A third limitation is called low **perfusion**. Pulse oximetry is based on having a pulsatile signal. If the pulsatile signal is small compared with the DC signal, usually 1 count in 1000, the *R* value calculation becomes inaccurate. There are two reasons for the inaccuracy, round-off error and resolution. The round-off error can be compensated for by using more precision during the *R* value calculations.

Another limitation, which is difficult to eliminate, is motion artifact and autonomic nervous system response. Whenever there is an autonomic nervous system action, there is a transition or movement in the pulse oximetry signal. The most common technique to correct for motion artifact is averaging of consecutive measurements. Averaging works, but it slows the response time and lengthens the processing time. Another improvement would be to identify and eliminate inaccurate readings. This approach, however, is difficult to implement [7,21–23].

Reflectance Oximetry

An alternative technique to consider for DPP is reflectance probe measurements, as shown in Figure 78.10. Reflectance pulse oximetry has been addressed for *in vivo* studies by Mendelson and Ochs [24]. Because reflectance probes can be placed flat on the measurement area, they provide better shielding than a probe

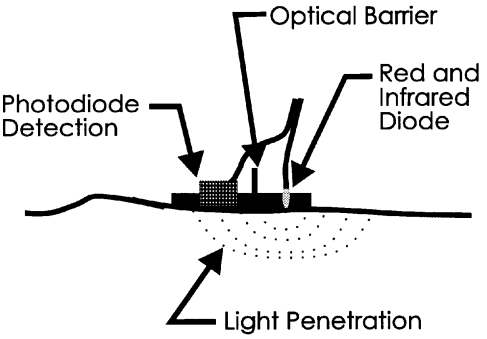

FIGURE 78.10 Reflectance oximetry probe. Red and infrared diodes illuminate the surface alternately and the reflected light is detected in close proximity by a photodiode. An optical barrier is required to eliminate direct light detection.

placed across a finger. However, reflectance measurements have less intensity than transmission measurements. Reflectance probe location and temperature effects have been analyzed. Increased temperature results in perfusion and increased signal intensity.

Schmidt et al. [25] reported an integrated circuit–based optical sensor for *in vivo* surface measurements. A set of equations was derived to estimate the amount of reflectance at a given intensity. The solution is a three-wavelength reflectance probe, with a red LED wavelength emission of 660 nm and a near and far infrared LED. The reflectance depth was calculated to be within 2 mm of the surface of the probe. The third wavelength is used to eliminate errors in measurement due to additional hemoglobin derivatives. The third wavelength was used to calculate a hematocrit value. The saturation was calculated using Equation 78.14.

$$SO_2 = A - B\left(R_{805} / R_{660}\right) \tag{78.14}$$

A and *B* are constants empirically derived for a specific light source and detector under specific physiological conditions.

A current method to measure S_vO_2 is by reflectance oximetry methods. A bundled fiber-optic cable is placed through a catheter. Light enters by a fiber or fibers and the reflected light is analyzed for oxygen content using spectrophotometry. The probe is similar to the fluorescence probe shown in Figure 78.4 with no chemistry envelope. The reflectance is used to determine the oxygen concentration empirically [24–26].

pH Measurements

Blood pH is very important in sustaining life. There is a small range of values allowable to maintain life, and pH is one of the most tightly regulated parameters in the body. Typical pH measurements are take invasively either through a catheter probe or as a blood sample measurement. pH is noted as the negative decade logarithm of the molal activity of hydrogen ions. The acid and alkaline hydrogen reactions are given in Equations 70.15 and 70.16, respectively.

$$O_2 + 4H^+ + 4e \rightarrow 2H_2O \tag{78.15}$$

$$O_2 + 2H_2O + 4e \rightarrow 4OH^- \tag{78.16}$$

The pH level can be determined by electrochemical means. The original pH measurements were performed using hydrogen electrodes after the oxygen (O_2) was eliminated. Current electrochemical measurements use a glass electrode sensitive to hydrogen and a concentrated KCl bridge.

Optical measurements of pH are performed fluorescently. It was determined that pH corresponds to CO_2 tension. Both PCO_2 and pH can be measured using a dye sensitive to hydrogen ions and using a variant of the Henderson–Hasselbalch equation, shown in Equation 78.17.

$$I_0 / I = 1 + K * H^+ \tag{78.17}$$

I is the relative fluorescence intensity, H^+ is the hydrogen ion concentration, and I_0 and *K* are calibration constants.

pH fluorescence measurements can be obtained using a pH-sensitive dye, such as phenol red. The basic form of phenol red is green absorbing, and the acidic form is blue absorbing. Exciting the phenol red buffer with a green 560 nm and red 600 nm light, the ratio of intensities can be used to calculate the pH according to Equation 78.18.

$$R = k * 10^{\left[-C / \left(10^{-\delta} + 1\right)\right]} \tag{78.18}$$

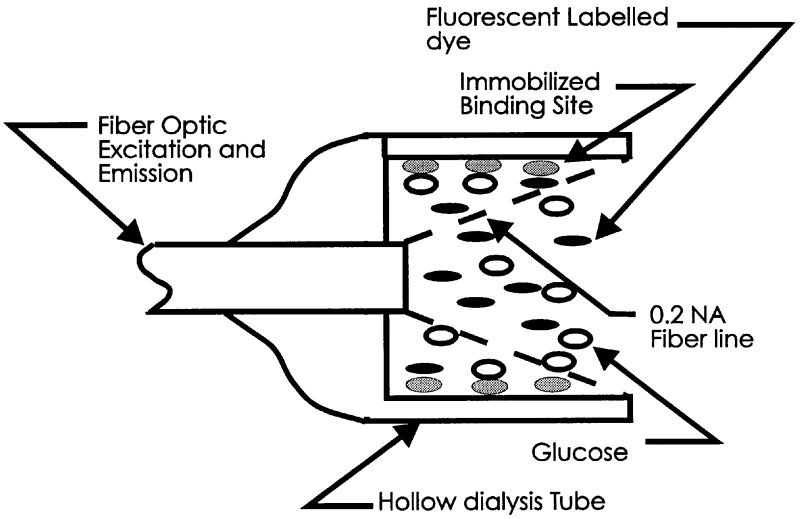

FIGURE 78.11 Optical glucose probe. A hollow tube is used to localize fluorescent dye outside the numerical aperture of the fiber. The fiber is used both to introduce excitation light and to collect emitted fluorescent levels. Only dye in the fiber view is illuminated. Dye bound to the hollow tube is not illuminated and is not detected.

k is the system optical constant, C is the green base form intensity, and δ is the difference between the pH and pK of the dye. Intensity shifts are accounted for by measuring the difference in intensity of two different excitation wavelengths. An alternative two-wavelength approach using hydroxypyrene trisulfonic acid (HTA) can be used. The basic form of HTA has a maximum excitation at a wavelength of 460 nm and an acidic maximum excitation wavelength of 410 nm. The ratio of the two fluorescent intensities at 520 nm is used to calculate the pH value [2,6,16,27].

Glucose Measurement

A common **glucose** measurement technique is by enzymatic amperometric measurements. Through selective binding, glucose and other compounds can be measured. The bound glucose changes the conductance and the glucose concentration can be determined.

Another more recent and interesting measurement technique is enzymatic optical measurements. Competitive binding between glucose and a fluorescein-labeled analog is used to determine glucose concentrations. An immobilized binding site is used to bind a fluorescent material, such as fluorescein-labeled dextran. A diagram of a glucose sensor is shown in Figure 78.11. The binding sights are fixed outside the excitation illumination. As the glucose concentration increases, the bound fluorescent material is released into the optical path. As the concentration of glucose increases, the viewed fluorescence increases and the reflected intensity increases. The reflected intensity will follow the concentration of glucose, and the actual response is measured empirically. The probe operation is reversible, allowing the probe to be reused. Reversibility is a requirement for implantable probes, which is the intended application for the optical glucose probe [27,28].

Electrolyte Concentration Measurements

In the clinical setting, it is important to monitor the concentration of various **electrolytes**. Typical measurements include sodium (Na^+), calcium (Ca^{++}), potassium (K^+), chloride (Cl^-), and magnesium (Mg^{++}). The concentrations are typically invasively drawn samples measured in a clinical laboratory. The measurement is performed using an ion-specific electrode, which is similar to the Clark electrode described previously. The clinical significance of different electrolytes is outlined in the text by

McClatchey, *Clinical Laboratory Medicine* [9]. Optical measurement techniques have been performed, but are not typically used in the clinical setting and are not covered here.

78.4 Combined Analysis Techniques

There are many combinations of instruments available. As instruments become more accurate and smaller, the trend is to simplify measurements and increase usefulness of equipment. Several selected instruments are described which have combined blood chemistry measurements into one unit.

CO-Oximetry

CO-oximetry has been noted for some time as the gold standard for oximetry readings. The more typical CO-oximeter does not measure just oxygen saturation, but several hemoglobin concentrations. For example, the Instrumentation Laboratory, Inc., IL 282 CO-oximeter uses an invasively drawn sample and measures total hemoglobin, oxyhemoglobin, carboxyhemoglobin, methemoglobin, and oxygen content in blood. The measurement technique is optical absorption. Four wavelengths at 535, 585.2, 594.5, and 626.6 nm are generated using a hollow cathode lamp. The extinction coefficients are used to calculate the concentration of RHb, O_2Hb, COHb, and MetHb. The total hemoglobin (tHb) concentration is calculated as the sum of the individual hemoglobin groups. The oxygen saturation (S_aO_2) is calculated as the concentration of oxyhemoglobin divided by the total concentration of hemoglobin. The total hemoglobin concentration, however, is usually noted as only the oxyhemoglobin concentration divided by the concentration of reduced hemoglobin and oxyhemoglobin. Therefore, care must be used in comparisons, because variations in S_aO_2 calculations can occur. The oxygen content is calculated as 1.39 times the concentration of oxyhemoglobin [29].

Intra-Arterial Probes

Intra-arterial probes have drawn much interest. They allow continuous or periodic measurements in the clinical setting in real time. These probes also offer minimally invasive measurements. The intent is to use intraarterial measurements on patients who require catheterization. The goal is a small probe that can be inserted into a catheter and not interfere with administering therapies or blood sampling. An optode-type probe is described by Shapiro et al. [30,31]. A fiber-optic probe is described by Gehrich et al. [15] for blood gas monitoring. The probe is designed to measure pH, PCO_2, and PO_2. In addition, a thermocouple is used to adjust for temperature variations, and values are normalized to standard temperatures. The sample drawing of the probe is shown in Figure 78.12. A set of chemical optical fluorescent probes is used.

78.5 Evaluation of Technology

There have been numerous measurement types and techniques used in developing an understanding of blood chemistry. The diverse background has resulted in very good indicators of how blood is used to sustain life. Despite the diverse background, many changes and advances are occurring at an impressive rate. Immediate improvements are being tested and implemented on a regular basis, and novel approaches will be seen in the near future. In addition, there is an explosion of studies being performed to understand how the body works and is put together. A brief overview of some improvements that can be expected follows.

Some basic engineering changes could be performed to enhance accuracy. Oximetry measurements could easily see engineering improvements. New techniques have been tested, but approval and acceptance are slow. There has been progress made in size, with new oximeters that can clip on the finger. The accuracy has seen only moderate improvement. Alternative probes and algorithms have been tested and even patented, but have not been marketed. One example of a patent is for Fourier analysis of pulse

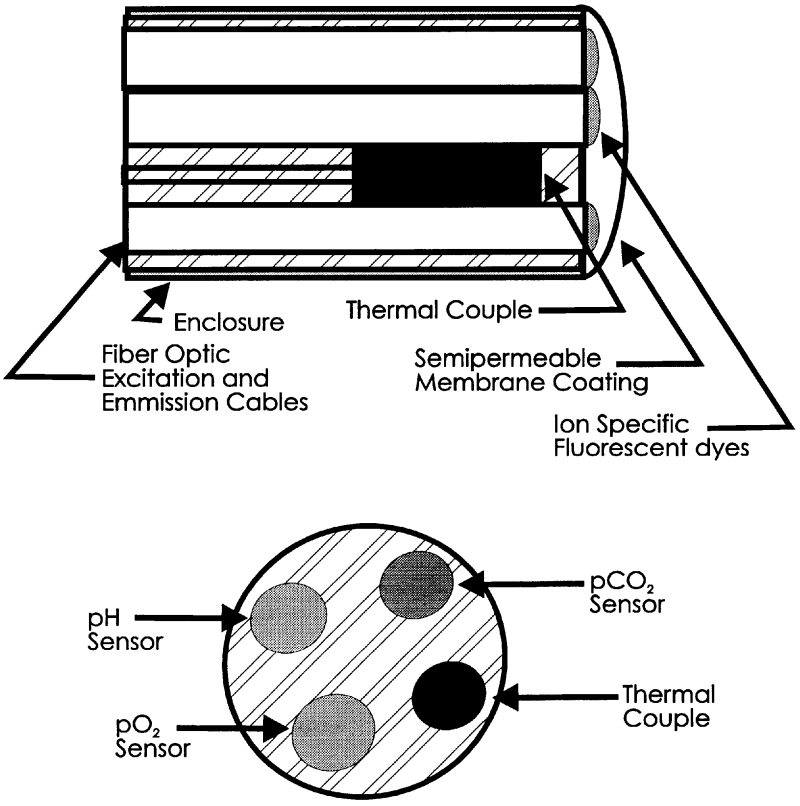

FIGURE 78.12 Combined fiber-optic sensor for pH, PCO_2, PO_2, and temperature correction measurements. Each sensor is bundled into a single enclosure and has separate detection areas.

oximetry signals for arterial saturation measurements. The spectral analysis would allow the possibility to implement some novel filter and analysis routines. This would improve stability from motion artifact. Hardware changes could also bring changes. Resolution could be improved by using more accurate photodiodes and A/D converters, or by using laser diodes for more intense and accurate light illumination. Reflectance probe advances would improve versatility.

The future of blood chemistry should see continued miniaturization. Fiber optics have become a major research direction. Fiber optics allow remote sensing and have reduced the possibility of electrical and magnetic complications for patient monitoring. Miniature fiber-optic sensors are being developed for numerous applications, and the improvement and developments can be applied to biological readings. Miniaturization not only reduces size, but can also improve stability. Small probes also allow multiple readings in one sensor. This trend has already started with blood gas probes being used for intravascular and extravascular measurements. The next trend envisioned is nanoprobes. Implantable glucose probes are already being tested. Sensor and light sources can be implemented on an integrated circuit and continue to shrink. Microsensors are already being used in biomedical application, such as blood pressure sensors and single-cell neural stimulators. Miniature chemistry and thermal-cycling laboratories are being developed into single integrated chips. The same chemical and optical arrangements can be performed for blood chemistry analysis. A complete laboratory measurement system could be at the patient location for continuous monitoring [28–30,32].

The other major anticipated change is the trend for additional and more accurate noninvasive readings. Invasive measurements can mean delays and noncontinuous monitoring, as well as increased possibility of infection. Given equal measurement accuracy, noninvasive measurements are an obvious choice over invasive readings.

It is important to note that measurement techniques will continue to improve, but the rate of growth and utilization will not grow as fast. Acceptance and utilization are dependent on cost and the effort involved in using the instruments. Cost can slow or even eliminate instrumentation from being used. However, with the increasing cost of medical care, there is interest in providing fast diagnosis and recovery for patients. This will provide incentive for improving measurement technique and accuracy.

Defining Terms

Artery: Vessel used to carry blood away from the heart.
Capillary: Semipermeable membrane use to exchange oxygen and other substances with tissue.
Catheter: Surgical instrument inserted into the body for drawing or administering fluids.
Electrolyte: A substance that dissolves into ions, becoming capable of conducting electricity.
Fluorescence: The property of emitting light when exposed to an excitation light.
Glucose (dextrose): Substance found in normal blood, which is the main source of energy in living tissue.
Hematocrit: The total concentration of hemoglobin, or packed red cells, in blood.
Hemoglobin: Oxygen-carrying pigment found in blood, which has the property of reversible oxygenation.
Intra-arterial: Within the artery.
Invasive: Pertaining to the insertion of an instrument into the body.
Oximetry: Photoelectric determination of arterial blood oxygen saturation (S_aO_2).
Perfusion: Pertaining to the passage of fluid through vessels. Low perfusion refers to reduced blood flow.
Photoplethysmogram: Chart of the volume change in an organ or limb. In oximetry it is the chart of the light passed through an ear, nose, or digit. The light intensity varies with the arterial pulse, which is related to the arterial volume.
Transcutaneous: Pertaining to entering through the skin.
Vein: Vessel used to return blood to the heart.

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