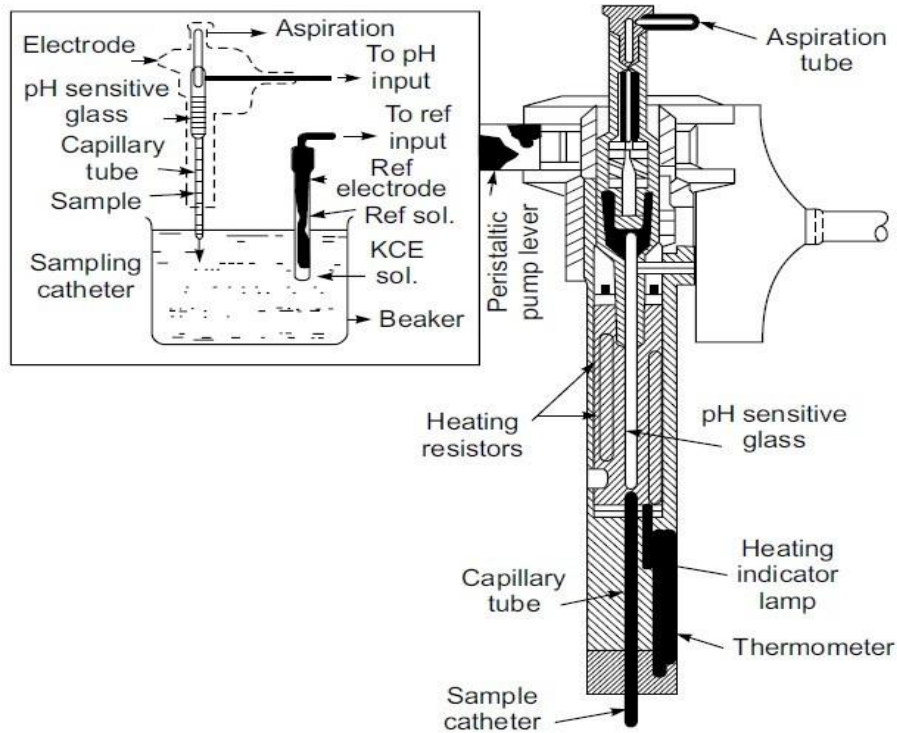


PH of Blood

- The pH scale, otherwise known as the acid-base scale, runs from 0 to 14. It measures how acidic a solution of a substance in water is. For example, pure water has a pH of 7.
- Solutions with a low pH have a high concentration of hydrogen ions and are acidic. Solutions with a high pH have a lower concentration of hydrogen ions and are alkaline, or basic.
- The pH scale is a compact scale, and small changes in pH represent big leaps in acidity.
- Electrodes for Blood pH Measurement: Several types of electrodes have been described in literature for the measurement of blood pH. They are all of the glass electrode type but made in different
- Typically, a micro-electrode for clinical applications requires only 20–25 μl of capillary blood for the determination of pH. The electrode is enclosed in a water jacket with circulating water at a constant temperature of 38°C. The water contains 1% NaCl for shielding against static interference. The capillary is protected with a polyethylene tubing. The internal reference electrode is silver/silver chloride and the calomel reference electrode is connected to a small pool of saturated KCl, through a porous pin. An accuracy of 0.001 pH can be obtained with this electrode against a constant buffer
- Quite often, combination electrodes comprising both measuring and reference electrodes offer single-probe convenience for all pH measurements.
- Several instruments offer the ability to measure pH in small containers with as little as 250 μl of the sample
- The pH of blood is found to change linearly with temperature in the range of 18° to 38°C.
- The temperature coefficient for the pH of blood is 0.0147 pH unit per degree centigrade. This necessitates the use of a highly accurate temperature-controlled bath to keep the electrodes with the blood sample at 37°C ± 0.01°C.
- Buffer Solutions: Buffer solutions are primarily used for (i) creation and maintenance of a desired, stabilized pH in a solution and (ii) standardization of electrode chains for pH measurements. A buffer is, therefore, a substance which by its presence in a solution is capable of counteracting pH changes in the solution as caused by the addition or the removal of hydrogen ions. Buffer solutions are characterized by their pH value.



Microcapillary electrode for measurement of blood pH (Courtesy: Corning)

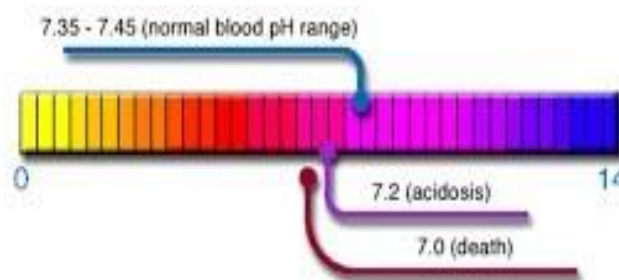
Normal blood pH levels

The pH of blood in the arteries should be between **7.35 and 7.45** for the body's metabolic processes and other systems to work well.

Changes in pH blood level

The pH of the blood can change in both directions.

Acidosis occurs when the blood is too acidic, with a pH below 7.35. Alkalosis occurs when the blood is not acidic enough, with a pH above 7.45.



There are four main ways in which blood pH can change:

- **Metabolic acidosis:** This occurs due to reduced bicarbonate or increased acid levels.
- **Respiratory acidosis:** This occurs when the body removes less carbon dioxide than usual.
- **Metabolic alkalosis:** This occurs due to increased bicarbonate or reduced acid levels.
- **Respiratory alkalosis:** This occurs when the body removes more carbon dioxide than usual.

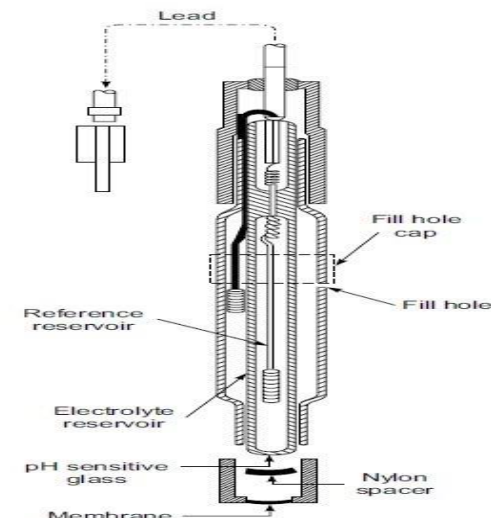
Measurement of Blood pCO₂, pO₂

Measurement of pCO₂

- The blood pCO₂ is the partial pressure of carbon dioxide of blood taken anaerobically. It is expressed in mmHg and is related to the percentage CO₂ as follows:

$$p\text{CO}_2 = \text{Barometric pressure} - \text{water vapour pressure} \times \frac{\% \text{CO}_2}{100}$$

- All modern blood gas analysers make use of a pCO₂ electrode. It basically consists of a pH sensitive glass electrode having a rubber membrane stretched over it, with a thin layer of water separating the membrane from the electrode surface.
- The technique is based on the fact that the dissolved CO₂ changes the pH of an aqueous solution. The CO₂ from the blood sample diffuses through the membrane to form H₂CO₃, which dissociates into (H⁺) and (HCO⁻₃) ions. The resultant change in pH is thus a function of the CO₂ concentration in the sample. The emf generated was found to give a linear relationship between the pH and the negative logarithm of pCO₂.



➤ Fig. 15.3 Construction of pCO₂ electrode (Courtesy: Corning Scien-

- The basic construction of the electrode was modified to a degree that made it suitable for routine laboratory use. In the construction worked out by them, the water layer was replaced by a thin film of an aqueous sodium bicarbonate (NaHCO₃) solution. The rubber membrane was also replaced by a thin Teflon membrane, which is permeable to CO₂ but not to any other ions, which might alter the pH of the bicarbonate solution. The CO₂ from the blood diffuses into the bicarbonate solution. There will be a drop in pH due to CO₂ reacting with water forming carbonic acid. The pH falls by almost one pH unit for a ten-

fold increase in the CO₂ tension of the sample. Hence, the pH change is a linear function of the logarithm of the CO₂ tension.

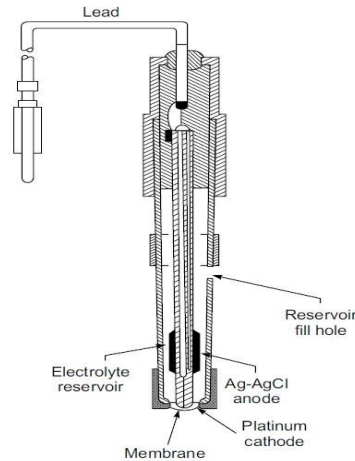
- Further improvements in stability and response time. They used a dilute solution of NaHCO₃ (0.0001 N), which helped in reducing the response time but the drift introduced posed serious problems. The compromise between response time and drift was achieved by using a 0.001 N solution of NaHCO₃. Silver/silver chloride reference electrode was replaced by a calomel cell which was made an integral part of the electrode
- Further improvement constructed a pCO₂ electrode using 0.5 mm polyethylene as a membrane and used no separator between the glass surface and this membrane. They added carbonic anhydrase to the electrolyte. The response time was found to be 6 seconds for 90% of a step change from 2% to 5% CO₂. Use of a pCO₂ electrode for the measurement of blood or plasma pCO₂ has been studied repeatedly and has been found to be accurate, precise and expedient.
- The emf generated by a pCO₂ electrode is a direct logarithmic function of pCO₂. It is observed that a ten-fold change in pCO₂ causes the potential to change by 58 ± 2 mV. The pH versus log pCO₂ relationship is linear within ± 0.002 pH unit from 1 to 100% carbon dioxide.
- It is essential to maintain the temperature of the electrode assembly constant within close limits.
- The combined effects of temperature change upon the sensitivity of the pH electrode and upon the pCO₂ of the blood sample amount to a total variation in sensitivity of 8% per degree centigrade.
- Calculated Bicarbonate, Total CO₂ and Base Excess: Acid-base balance determinations are based on several calculations, which are routinely used in conjunction with blood pH and gas analysis. An accurate picture of acid-base balance can be determined from the equilibrium:

Measurement of pO₂

The partial pressure of oxygen in the blood or plasma indicates the extent of oxygen exchange between the lungs and the blood, and normally, the ability of the blood to adequately perfuse the body tissues with oxygen. The partial pressure of oxygen is usually measured with a polarographic electrode. There is a characteristic polarizing voltage at which any element in solution is predominantly reduced and in the case of oxygen, it is 0.6 to 0.9 V. In this voltage range, it is observed that the current flowing in the electrochemical cell is proportional to the oxygen concentration in the solution.

Most of the modern blood gas analysers utilize an oxygen electrode for measuring oxygen partial pressure. This type of electrode consists of a platinum cathode, a silver/silver chloride anode in an electrolyte filling solution and a polypropylene membrane. The electrode is of a single unit construction and contains the reference electrode also in its assembly.

The entire unit is separated from the solution under measurement by the polypropylene

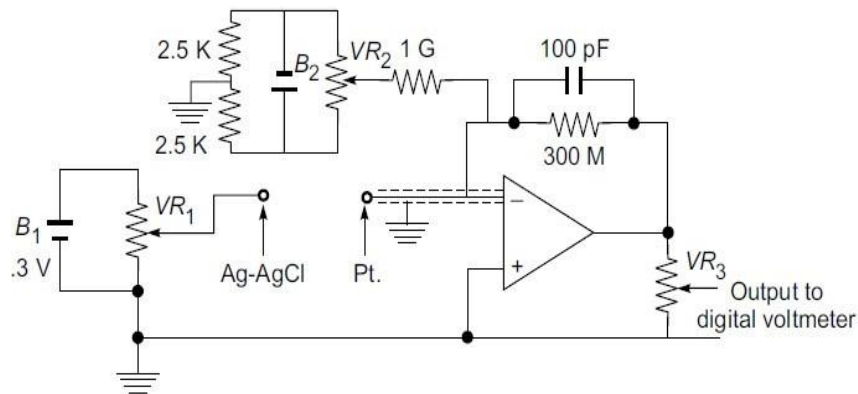


► Fig. 15.4 Constructional details of pO_2 electrode (Courtesy: Corning Scientific Instruments, U.S.A.)

membrane.

- Oxygen from the blood diffuses across the membrane into the electrolyte filling solution and is reduced at the cathode. The circuit is completed at the anode, where silver is oxidized, and the magnitude of the resulting current indicates the partial pressure of oxygen. The reactions occurring at the anode and cathode are:
- The Clark electrode for measuring pO_2 has been extensively studied and utilized. It is found to be of particular advantage for measuring blood samples.
- The principal advantages are:
 - (i) Sample size required for the measurement can be extremely small,
 - (ii) The current produced due to pO_2 at the electrode is linearly related to the partial pressure of oxygen,
 - (iii) The electrode can be made small enough to measure oxygen concentration in highly localized areas,
 - (iv) The response time is very low, so the measurements can be made in seconds.
- The ammonium hydroxide on the tip of the electrode (10% solution), with a gentle, rotary motion using a swab is used. The silver chloride gets dissolved in ammonium hydroxide. It is then flushed with distilled water.
- The polarographic electrodes usually exhibit ageing effect by showing a slow reduction in current over a period of time, even though the oxygen tension in the test solution is maintained at a constant level. Therefore, it needs frequent calibration.
- The measurement of current developed at the pO_2 electrode due to the partial pressure of oxygen presents special problems.

- Measurement of oxygen electrode current is made by using high input impedance, low noise and low current amplifiers. Field effect transistors usually form the input stage of the preamplifiers.



► Fig. 15.6 Current amplifier for use with pO_2 electrode

Increased pCO_2 is caused by:

- Pulmonary edema
- Obstructive lung disease

Decreased pCO_2 is caused by:

- Hyperventilation
- Hypoxia
- Anxiety

PO_2 (partial pressure of oxygen) reflects the amount of oxygen gas dissolved in the blood. It primarily measures the effectiveness of the lungs in pulling oxygen into the blood stream from the atmosphere.

Elevated pO_2 levels are associated with:

- Increased oxygen levels in the inhaled air
- Polycythemia

Decreased PO_2 levels are associated with:

- Decreased oxygen levels in the inhaled air
- Anemia
- Heart decompensation
- Chronic obstructive pulmonary disease
- Restrictive pulmonary disease
- Hypoventilation

ANALYTE	Normal Value	Units
pH	7.35 - 7.45	
PCO2	35 - 45	mm Hg
PO2	72 - 104	mm Hg`
[HCO3]	22 - 30	meq/L
SaO2	95-100	%
Anion Gap	12 \pm 4	meq/L
Δ HCO3	+2 to -2	meq/L