Blood Gas Analysers

Importance

Blood gas analyzers are used to measure the pH, partial pressure of carbon dioxide (pCO2) and partial pressure of oxygen (pO2) of the body fluids with special reference to the human blood. The measurement of these parameters are essential to determine the acid-base balance in the body. A sudden change in the pH and pCO2 could result in cardiac arrhythmias, ventricular hypotension and even death. This shows the importance of the maintenance of physiological neutrality in blood, and consequently the crucial role that the blood gas analyzers play in clinical medicine

Acid Base Balance

- The normal pH of the extracellular fluid lies in the range of 7.35 to 7.45, indicating that the body fluid is slightly alkaline. When the pH exceeds 7.45, the body is considered to be in a state of alkalosis. A body pH below 7.35 indicates acidosis. Both acidosis or alkalosis are disease conditions widely encountered in clinical medicine. Any tendency of the pH of blood to deviate towards these conditions is dealt with by the following three physiological mechanisms: (i) buffering by chemical means, (ii) respiration, (iii) excretion, into the urine by kidneys
- The blood and tissue fluids contain chemical buffers, which react with added acids and bases and minimize the resultant change in hydrogen ions.
- **Blood respond to changes in carbon dioxide concentration in seconds.** The respiratory system can adjust sudden changes in carbon dioxide tension back to normal levels in just a few minutes. Carbon dioxide can be removed by increased breathing and therefore, hydrogen concentration of the blood can be effectively modified.
- **The kidney requires many hours to readjust hydrogen ion concentration by excreting highly acidic or alkaline urine to enable body conditions to return towards normal.**

Acid Base Balance Continued..

Arterial blood has a pH of approximately 7.40. As venous blood acquires carbon dioxide, forms carbonic acid and hydrogen ions, the venous blood pH falls to approximately 7.36. This pH drop of 0.04 units occurs when the $\mathbf{CO_2}$ enters the tissue capillaries. When $\mathbf{CO_2}$ diffuses from the pulmonary capillaries into the alveoli, the blood pH rises 0.04 units to bring the normal arterial value of 7.40. It is quite difficult to measure the pH of fluids inside the tissue cells, but from estimates based on $\mathbf{CO_2}$ and $\mathbf{HCO_3^-}$ and H^+ ion concentration, intracellular pH probably ranges from 7.0 to 7.2.

In order to maintain p$\mathbf{O_2}$, p$\mathbf{CO_2}$ and pH within normal limits, throughout the wide range of body activity, the rate and depth of respiration vary automatically with changes in the metabolism. Control of alveolar ventilation takes place by means of chemical as well as nervous mechanisms. The three important chemical factors regulating alveolar ventilation are the arterial concentrations of $\mathbf{CO_2}$, H+ and $\mathbf{O_2}$. Carbon dioxide tension in the blood stream and cerebrospinal fluid is the major chemical factor regulating alveolar ventilation. The carotid and aortic chemoreceptors stimulate respiration when oxygen tension is abnormally low. There are many organs that participate in the control of respiration.

Typical Values of Blood Gas Parameters

• **Table 15.1** *Typical Expected Values of Blood Gas Parameters*

Parameter		*Arterial or arterialized capillary blood*	*Venous plasma (separated at 37 °C)*
pH		7.37 to 7.44	7.35 to 7.45
pCO_2	men	34 to 35 mmHg	36 to 50 mmHg
	women	31 to 42 mmHg	34 to 50 mmHg
pO_2	resting adult	80 to 90 mmHg	25 to 40 mmHg
	resting adult over 65 years	75 to 85 mmHg	
Biocarbonate	men	23 to 29 mmol/l	25 to 30 mmol/l
	women	20 to 29 mrnol/l	23 to 28 mmol/1
Total CO_2	men	24 to 30 mmol/l	26 to 31 mmol/l
(plasma)	women	21 to 30 mmol/l	24 to 29 mmol/l
Base Excess	men	–2.4 to +2.3 mmol/l	0.0 to +5.0 mmol/l
	women	–3.3 to +1.2 mmol/l	–1.0 to + 3.5 mmol/l

Blood pH Measurement

- The acidity or alkalinity of a solution depends on its concentration of hydrogen ions. Increasing the concentration of hydrogen ions makes a solution more acidic, decreasing the concentration of hydrogen ions makes it more alkaline. The amount of hydrogen ions generally encountered in solutions of interest is extremely small and, therefore, the figure is usually represented in the more convenient system of pH notation. pH is thus a measure of hydrogen ion concentration, expressed logarithmically.
- Specifically, it is the negative exponent (log) of the hydrogen ion concentration. $pH = -\log (H+)$
- If the hydrogen ion concentration increases then the pH falls and when the hydrogen ion concentration falls the pH increases

pH

Since pure water dissociates into $\mathbf{10^{-7}}$ mol/l of (H+) and $\mathbf{10^{-7}}$ mol/l of (OH–), a pH of +7 is considered a neutral l l solution; a pH of +6 represents an acid, a pH of +8 an alkali. Since $\mathbf{10^{-6}}$ is a larger number than $\mathbf{10^{-8}}$ former solution has a larger hydrogen ion concentration. Thus, a pH of 6 is more acidic than a pH of 8. Concentration of ions, like the concentration of atoms or molecules, is

expressed in terms of mols/l (1 mol = $\mathbf{6.02 * 10^{23}}$ molecules, known as the Avogadro's Number.

This is the number of molecules in one mole-an amount of material in grams equal to the molecular weight). Whole blood with a (H+) of 4 * $\mathbf{10^{-8}}$ moles/l would have a pH of 7.4; an

increase in the (H+) to 1 * $\mathbf{10^{-7}}$ moles/l would correspond to a decrease in pH to 7.0.

pH Measurement

Glass Electrode: Where the glass membrane separates two solutions with different pH. The reference solution pH is held constant, while the pH of another solution varies depending on the concentration resulting in a potential difference. This potential difference is measured using milli-voltmeter.

Nernst Equation : $$E = E_0 - \frac{2.3036\ RT}{F}\Delta pH$$

E= Measured Potential, E_o= Standard Potential

RT= Gas Constant , F= Faraday Constant, ΔpH = Difference pH

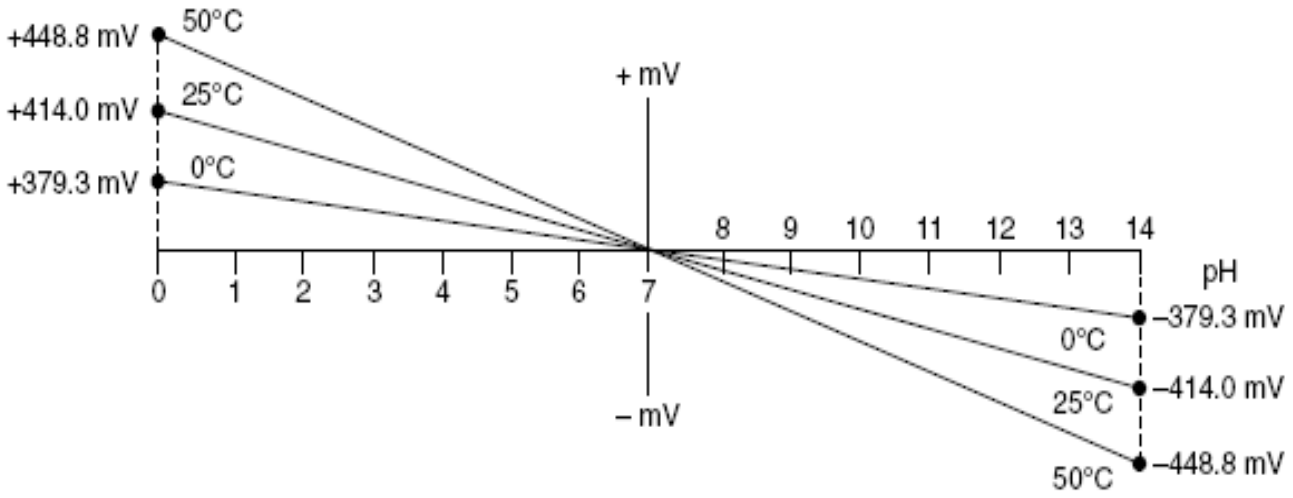
The equation shows that the potential developed is linearly proportional to the difference in pH

Continued…

Change of pH of one unit = 58.2 mV at 20°C = 62.2 mV at 40°C

And the slope factor (2.3036RT/F) is a temperature dependent factor

With a 1°C change in temperature, the emf changes by 0.2 mV



➤ **Fig. 15.1** *Relationship between pH and emf at different temperatures (Courtesy: Beckman Instruments Inc., USA)*

Continued.....

The solution is taken in a beaker. A pair of electrodes: one glass or indication electrode and the other reference or calomel electrode, are immersed in the solution. The voltage developed across the electrodes is applied to an electronic amplifier, which transmits the amplified signal to the display. The pH meter is usually equipped with controls for calibration and temperature compensation.

The glass electrode exhibits a high electrical resistance, of the order of 100–1000 MΩ. The emf measurement, therefore, necessitates the use of measuring circuits with high input impedance. Further, the high resistance of glass electrodes render them highly susceptible to capacitive pickup from ac mains. In order to minimize such effects, it is advisable to screen the electrode cable. The screen is usually grounded to the case of the measuring instrument

Calibration

The error caused in pH measurements due to temperature effect can be compensated either manually or automatically. In manual adjustment the instrument is calibrated at 25°C. Then the control is simply set to the actual measuring temperature. By this adjustment, the output current of the amplifier gets corrected to the desired temperature. In automatic adjustment, a variable resistor which is usually a thermistor or wire wound resistance that has an approximate desired resistance temperature coefficient is inserted in the circuit. During measurement, it is placed in the test solution. The use of an automatic temperature compensator will ensure that the pH meter is operating with a correct mV/pH conversion ratio.

If it is desired to have the accuracy of a pH measurement as 0.001 pH, then the voltage must be measured with an accuracy of 0.058 mV, assuming an ideal sensitivity of 58 mV per pH unit. With a symmetrical scale on the measuring device of 6 pH units around pH 7, the maximum voltage will be +_348 mv

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 shapes so that they may accept small quantities of blood and yield accurate results. The most common type is the syringe electrode, which is preferred for the convenience of taking small samples of blood anaerobically. The small 'dead space' between the electrode bulb and the inner surface of the syringe barrel is usually filled with dilute heparin solution to prevent blood coagulation. Before making measurements, the syringe should be rolled between the hands to ensure thorough mixing. Microcapillary glass electrodes are preferred when it is required to monitor pH continuously for example during surgery. These types of electrodes are especially useful when a very small volume of the sample is to be analyzed

- Typically, a micro-electrode for clinical applications requires only 20–25 ml 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% NACI 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 KCI, through a porous pin. An accuracy of 0.001 pH can be obtained with this electrode against a constant buffer.

Microcapillary Blood pH electrode

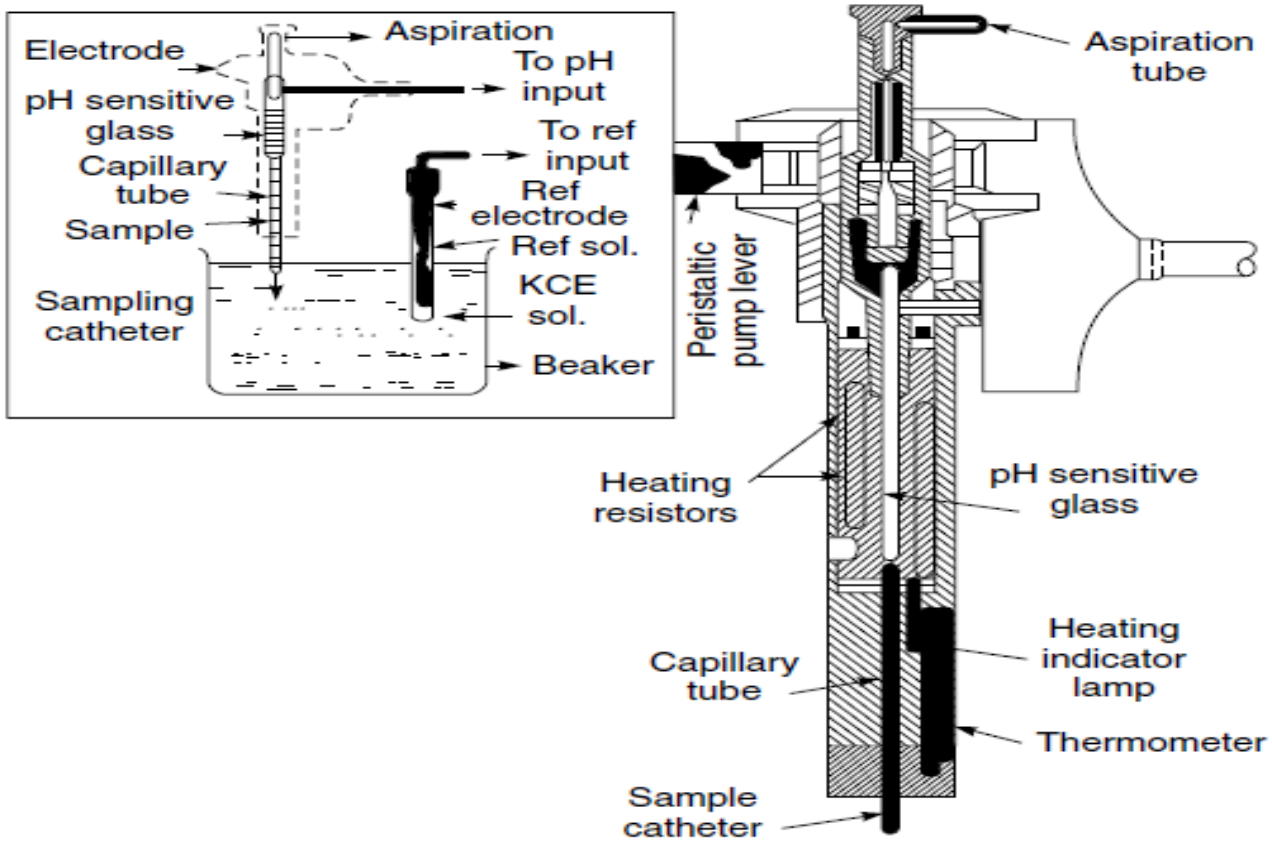


Fig. 15.2 *Microcapillary electrode for measurement of blood pH (Courtesy: Corning Scientific Instruments, USA)*

Macro electrodes are heavy due to the large size and the micro electrodes are very fragile.

A miniature pH glass electrode, using Corning 015 glass as the hydrogen-ion-sensitive glass. The dimensions of the electrode were 1.0 mm outside diameter and 0.25 mm wall thickness. The inner electrolyte is a solution of 0.1 N hydrochloric acid and the inner reference electrode is a silver/silver chloride electrode. The silver/silver chloride electrode is made from a silver wire (0.127 mm in diameter and 99.9% purity) by electrolytic method. A FET input operational amplifier is integrated into the pH electrode. Temperature response of the electrode was –1.51 mV/K at a pH value of 7. Evaluation of the stability of the electrode showed a 1% drift over a 7–hour operational time. The pH temperature hysteresis effects showed a 0.5 and 1.0% deviation, respectively. The response time was within 4s for a 99% response.

Measurement of Blood PCO_2

pCO_2

The blood pCO_2 is the partial pressure of carbon dioxide of blood taken anaerobically. It is expressed in mmHg and is related to the percentage CO_2 as follows:

pCO_2= (Barometric pressure – water vapour pressure)* (% pCO_2/100)

At 37°C (Normal blood temperature =98.6F), the water vapour pressure is 47 mmHg, so at 750 mm barometric pressure, 5.7% corresponds to a pCO_2 of 40 mm.

All modern blood gas analyzers make use of a pCO_2 electrode of the type described by Stow *et al* (1957). 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 CO_2 changes the pH of an aqueous solution. CO_2 from the blood sample difuses through the membrane to form H_2CO_3, which dissociates into H^+ and $H_2CO_3^-$ ions. The resultant change in pH is thus a function of the CO_2 concentration in the sample. The emf generated was found to give a linear relationship between the pH and the negative logarithm of pCO_2. Although the electrode could not provide sensitivity and stability required for clinical applications, it made way for realizing a direct method for the measurement of pCO2.

Severinghaus and Bradley Electrode

Modified electrode by Severinghaus and Bradley (1958) more suitable for a routine laboratory use.

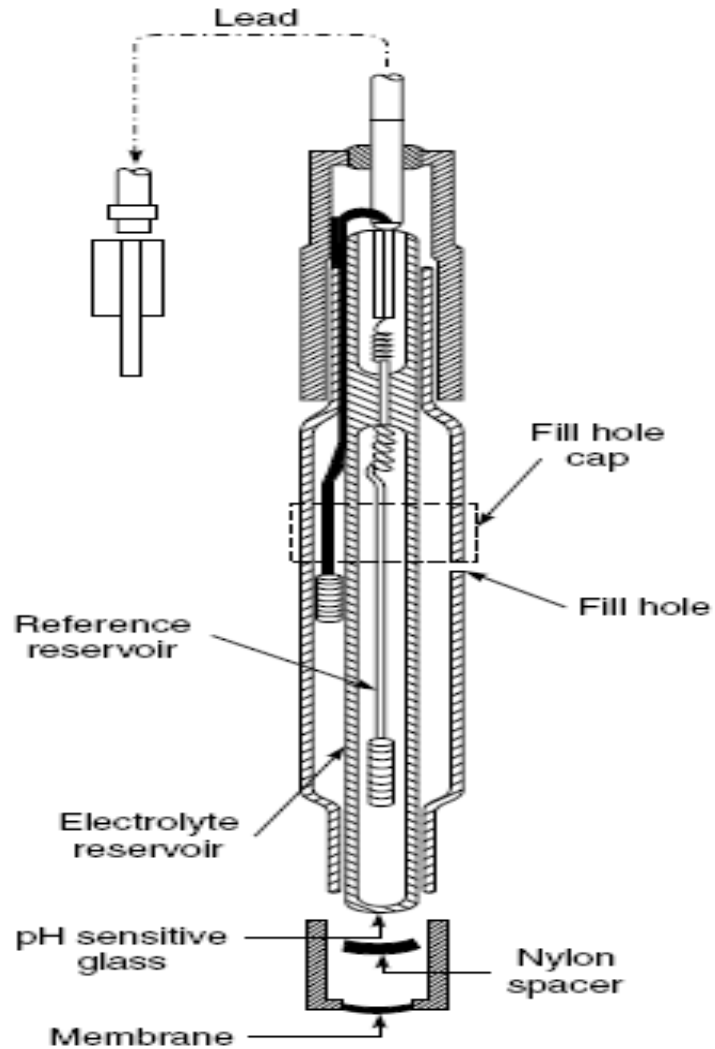
Construction : Water layer was replaced by a thin film of an aqueous sodium bicarbonate ($NaHCO_3$)solution. The rubber membrane was also replaced by a thin Teflon membrane, which is selectively permeable to CO_2 but not to any other ions, which might alter the pH of the bicarbonate solution. The CO_2 from the blood diffuses into the bicarbonate solution. There will be a drop in pH due to CO_2 reacting with water forming carbonic acid. The pH falls by almost one pH unit for a ten-fold increase in the CO_2 tension of the sample. Hence, the pH change is a linear function of the logarithm of the CO_2 tension. The optimum sensitivity in terms of pH change for a given change in CO_2 tension is obtained by using a bicarbonate solution of concentration of about 0.01 mole/l. The electrode is calibrated with the known concentration of CO_2. The response time of the CO_2 electrode is of the order of 0.5 to 3 min. This electrode was twice as sensitive and drifted much less than the Stow's electrode.

Hertz and Siesjo

Further improvements in stability and response time were achieved by Hertz and Siesjo (1959). They used a dilute solution of $NaHCO_3$ (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 $NaHCO_3$. Silver/silver chloride reference electrode was replaced by a calomel cell which was made an integral part of the electrode.

Improved Severinghaus

Severinghaus (1962) made a further improvement upon the earlier Severinghaus-Bradley electrode in the low pCO_2 range by replacing the cellophane spacer with a very thin nylon mesh. Glass fibres or powdered glass wool were also found to be good separators. He used a membrane of 3/8 mil Teflon and glass wool for the separator. Electrodes constructed in this way had a 95% response in 20s.



Construction of p CO_2 electrode (Courtesy: Corning Scientific Instruments, USA)

Performance Requirement of pH Meters

The emf generated by a pCO_2 electrode is a direct logarithmic function of pCO_2. It is observed that a ten-fold change in pCO_2 causes the potential to change by 58 ± 2 mV. The pH versus log pCO_2 relationship is linear within ±0.002 pH unit from 1 to 100% carbon dioxide.

1 pH=58 mV

0.001 pH=58 μv

For an accuracy of 0.1 mmHg, it is desirable to read 0.001 pH unit, i.e. a resolution of 58 ± 2 μv. This order of accuracy can be read only on a digital readout type pH meter or on an analog meter with expanded scale. The instrument should have a very high degree of stability and a very low drift amplifier. The input impedance of the electronic circuit must be at least $10^{12}\Omega$.

It is essential to maintain the temperature of the electrode assembly constant within close limits. It is experimentally shown that variation in the temperature of ± 1°C produces an error of ±1.5 mmHg . The combined effects of temperature change upon the sensitivity of the pH electrode and upon the pCO_2 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

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

$$H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

which for bicarbonate has an equilibrium constant

$$K_{H_2CO_3}/HCO_3^- = \frac{[H^+][HCO_3^-]}{H_2CO_3}$$

where (H^+), (HCO_3^-) and (H_2CO_3) refer to the concentration of these substances.

Since $H_2CO_3 = 0.03\ pCO_2$

and since $pH = -\log [H^+]$

Therefore, $$pH = pK + \log \frac{[HCO_3^-]}{0.03\ pCO_2}$$

where pK equals 6.11 for normal plasma at 37°C. This formula is used in blood gas analysers for calculating actual bicarbonate.

Total CO_2 is calculated from the relationship:

$$[HCO_3^-] + (0.03 \times pCO_2) = \text{total } CO_2 \text{ in millimoles/l}$$

Base excess is calculated from the formula described by Siggaard-Andersen (1963).

Base excess = $(1 - 0.0143 * Hb)\ [HCO_3^-] - (9.5 + 1.63\ Hb) * (7.4 - pH) - 24$

where Hb represents the patients' haemoglobin value.

Base excess is the number of milli equivalents of a strong acid or base which would be required per litre of blood to restore it to a pH of 7.400 at 37°C with pCO2 held at 40 torr (mm Hg). This is usually estimated from pH and pCO2 measurements done at 37°C in a sample of blood. Base excess of extracellular fluid is a quantity that reflects only the non-respiratory (metabolic) component of acid-base disturbances. It is the most used "non-respiratory" quantity for the diagnosis of acid-base disturbances and is calculated and presented by all blood gas analyzers produced today worldwide.

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Blood 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 analyzers utilize an oxygen electrode first described by Clark (1956) 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 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:

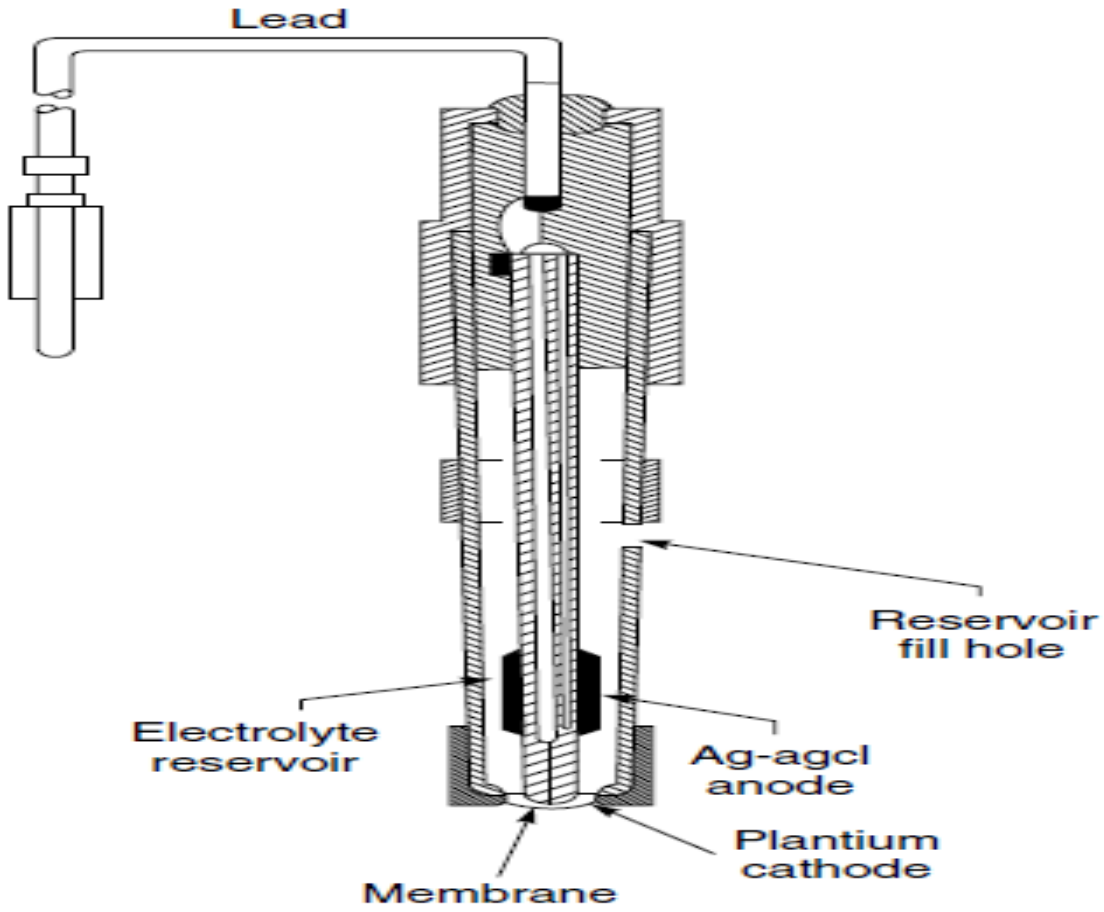
Cathode reaction:

$$O_2 + 2H_2O + 4e^- \rightarrow 4\,OH^-$$

Anode reaction:

$$4Ag \rightarrow 4Ag^+ + 4e^-$$

Clark's Electrode



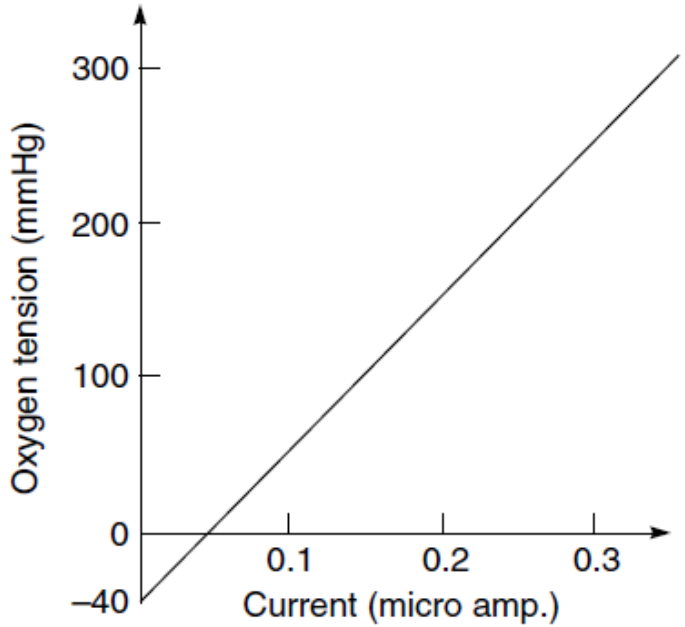
➤ **Fig. 15.4** *Construction of pO_2 electrode (Courtesy: Corning Scientific Instruments, USA)*

Advantages

(i) sample size required for the measurement can be extremely small,
(ii) the current produced due to pO2 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. As compared to this, it takes a very long time if the measurements are made by chemical means.

Calibration Curve

McConn and Robinson (1963) observed that zero electrode current was not given by a solution having zero Oxygen tension, but occurred at a definite oxygen tension, which they called the 'electrode constant'. So, for calibrating the electrode it was necessary to know this constant for that particular electrode. They further showed that when the straight line calibration curves (Fig. 15.5)
were extended backwards, they did not pass through the origin, but intersected the oxygen tension axis at a negative value. **To obtain a true zero-current (less than 10 nA), the electrolyte of the electrode is deoxygenated by bubbling nitrogen through it for about half an hour and then placing the electrode in water redistilled from alkaline pyragallol.**



➤ **Fig. 15.5** *Calibration curve of pO_2 electrode (after McConn and Robinson, 1963)*

Factors Affecting Measurement

The platinum cathode of the oxygen electrode tends to become contaminated or dimensionally unstable with time and use. The result is usually an inability to calibrate

and slope the electrode on any pO2 range. The manufacturers usually recommend application of ammonium hydroxide on the tip of the electrode (10% solution), with a gentle, rotary motion using a swab. 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. This is probably associated with the material depositing itself on to the electrode surface. The effect due to ageing can possibly be avoided by covering the electrode with a protective film of polyethylene, but it has the undesirable effect of increasing the response time.

- The measurement of current developed at the pO2 electrode due to the partial pressure of oxygen presents special problems. The difficulty arises because of the extremely small size of the electrical signal. The sensitivity (current per torr of oxygen tension) is typically of the order of 20 pA per torr for most commercial instruments. It is further subject to a constant drift and is also not independent of the sample characteristics. 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

Intra arterial Blood Gas Measurement

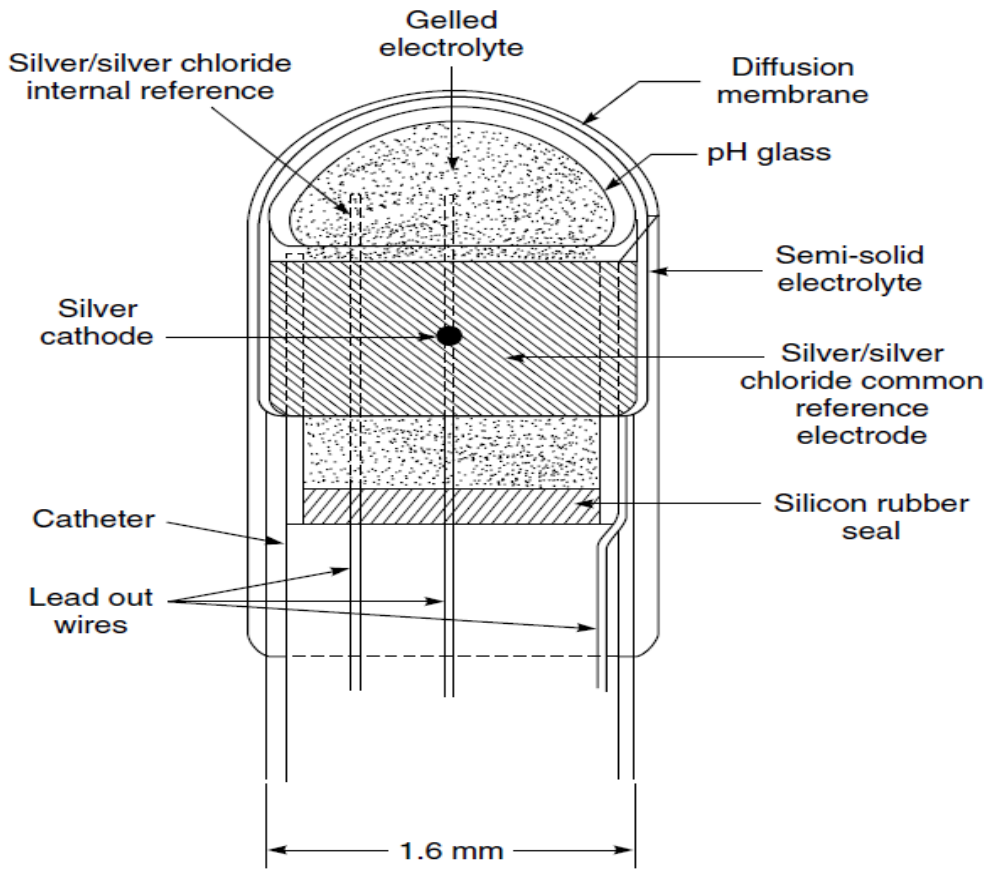
Need for in vivo Measurement

Arterial blood gas analysis is beneficial in the assessment and management of patients requiring mechanical ventilation and for those suffering from cardiopulmonary and other difficulties. Arterial blood gas values provide vital information about the adequacy of oxygenation, ventilation, acid-base balance and gas exchange in the lungs. *In vitro* blood gas analyzers, though commonly used, have several limitations. They require that blood be drawn and the sample analyzed, often at a distant blood gas laboratory. In addition to problems associated with blood handling, the need to send the sample to a laboratory delays results and treatment. Blood gas values can fluctuate rapidly in critically ill patients, and therefore, patient care decisions based on delayed information may be inappropriate. *In vivo* methods of blood gas monitoring have been developed to overcome these drawbacks.

Catheter Tip Electrode for Measurement of pO_2 & pCO_2

Miniature electrodes are required for *in vivo* transcutaneous measurements of pO_2 and pCO_2. The electrodes must be small enough to be mounted on the catheter pCO_2 tip and should preferably perform measurements of more than one parameter. One such electrode capable of simultaneous measurement of both pO_2 and pCO_2 is described by Parker *et al* (1978). The device is built into the tip of a SF (1.65 mm) catheter, 40 cm in length. The electrode (Fig. 15.7) comprises a pH sensitive glass bulb at the tip of the catheter for measuring changes in pH and hence pCO_2 according to the method escribed by Severinghaus and Bradley (1958).

A 180 mm diameter silver cathode constitutes a pO_2 measuring electrode



➤ **Fig. 15.7** *Catheter tip electrode for measurement of pO_2 and pCO_2 (after Parker et al 1978; reproduced by permission of Med. & Biol. Eng. and Comput.)*

pO_2 Measurement with Cutaneous electrode

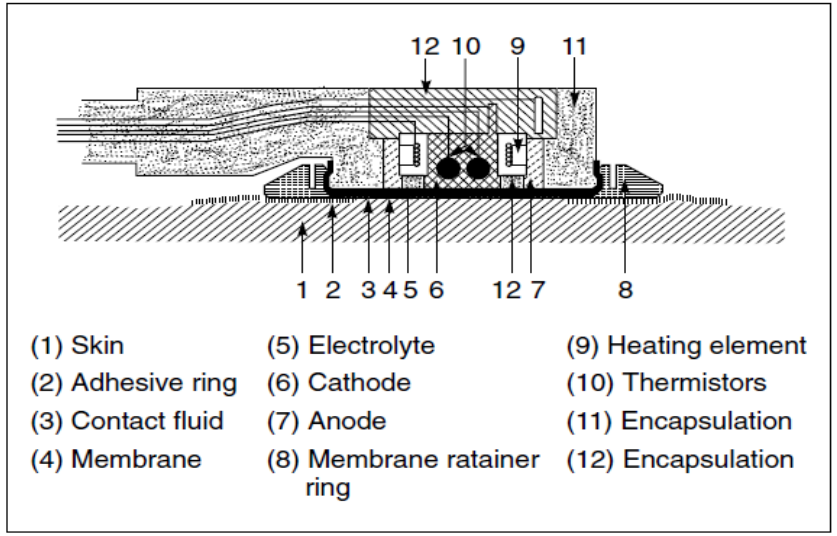
Continuous intra-arterial monitoring of oxygen is unsatisfactory as a clinically reliable procedure. It is expensive and relatively traumatic. Oximetric methods of monitoring oxygen tension from oxygen saturation are unreliable at the pO_2 level above 50 mmHg. Relatively simple and non-invasive methods are required to continuously monitor pO_2 to detect changes or establish trends. Eberhard *et al* (1973) concluded, after measuring oxygen tension using a Clark type electrode applied directly to the skin, that an excellent correlation (0.98) exists between skin pO_2 and arterial pO_2 in infants and new borns (Fig. 15.8). This could provide more immediate detection of hypoxia or hyperoxia than arterial sampling.

The principle underlying the skin sensor is that since oxygen is able to diffuse through body

tissue and skin, the measurement of pO_2 can be obtained indirectly by applying a Clark-type electrode sensor to the skin, heated to a constant temperature higher than the skin (44°C). Active vasodilation of the cutaneous vessels is achieved by warming the cathode and anode of the oxygen sensor to a temperature, which is higher than the normal body surface temperature.

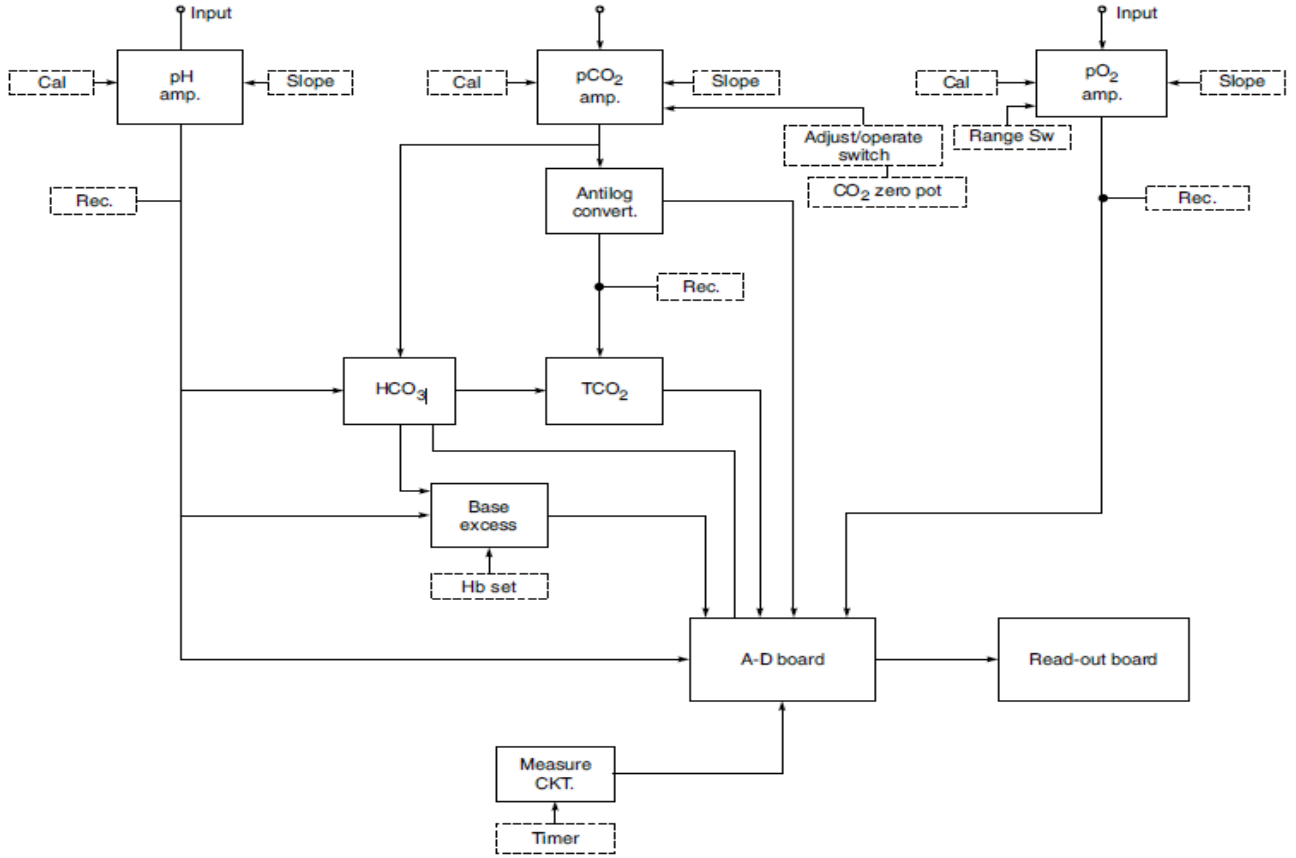
diffuses from the arterialized capillary bed through the epidermis to the skin surface and is measured there by an electrochemical reduction at the cathode of a Clark-type sensor. The electrode is 14 mm in diameter, with a 4 mm gold cathode, silver/silver chloride anode, covered with a 6 mm thick Mylar membrane (Fig. 15.9). The electrolyte used is a solution of KCI buffered to pH 10 which has a 3 to 45 days life if kept moist. A coil of resistance wire embedded in the Sensor heats the electrode to 44°C, a temperature selected to provide good hyperemisation as well as safety for continuous application over several days. A thermistor is also imbedded in the sensor to provide a control signal for monitoring constant temperature. The response time of the electrode is 60 s for 95% response to a full-scale step change. The sensor is applied to the skin using adhesive tape.

Cutaneous Oxygen Sensor



➤ **Fig. 15.9** *Cross-section of cutaneous oxygen sensor (Courtesy: Roche Medical Electronics Inc., USA)*

Complete Gas Analyser



➤ Fig. 15.10 *Block diagram of a complete blood gas analyser (Courtesy: Corning Scientific Instruments, USA)*

The complete gas analyser measures and gives a display of all the blood gas parameters of importance such as blood pH, pO2, pCO2, base excess and the concentration of bicarbonate ions. The block diagram shows the calibration of measured potential using the calibration curve slope adjustment to display in terms of the required parameter. The calibration has to be carried out with the known standard solutions at a given temperature. For pCO2 we observe a antilog block as the potential developed here is proportional to the negative logarithm of pCO2. The block diagram to calculate the base excess is given with the necessary Hb & HCO3 information.

ical equipment - Information

Blood Gas/pH/Chemistry Point of Care Analyzer

UMDNS		GMDN	
18853	Analyzers, Point-of-Care, Whole Blood, Gas/pH/ Electrolyte/Metabolite	56661	Blood gas analyser IVD, automated

Other common names:
POC Analyzer, blood gas analyzer

Health problem addressed

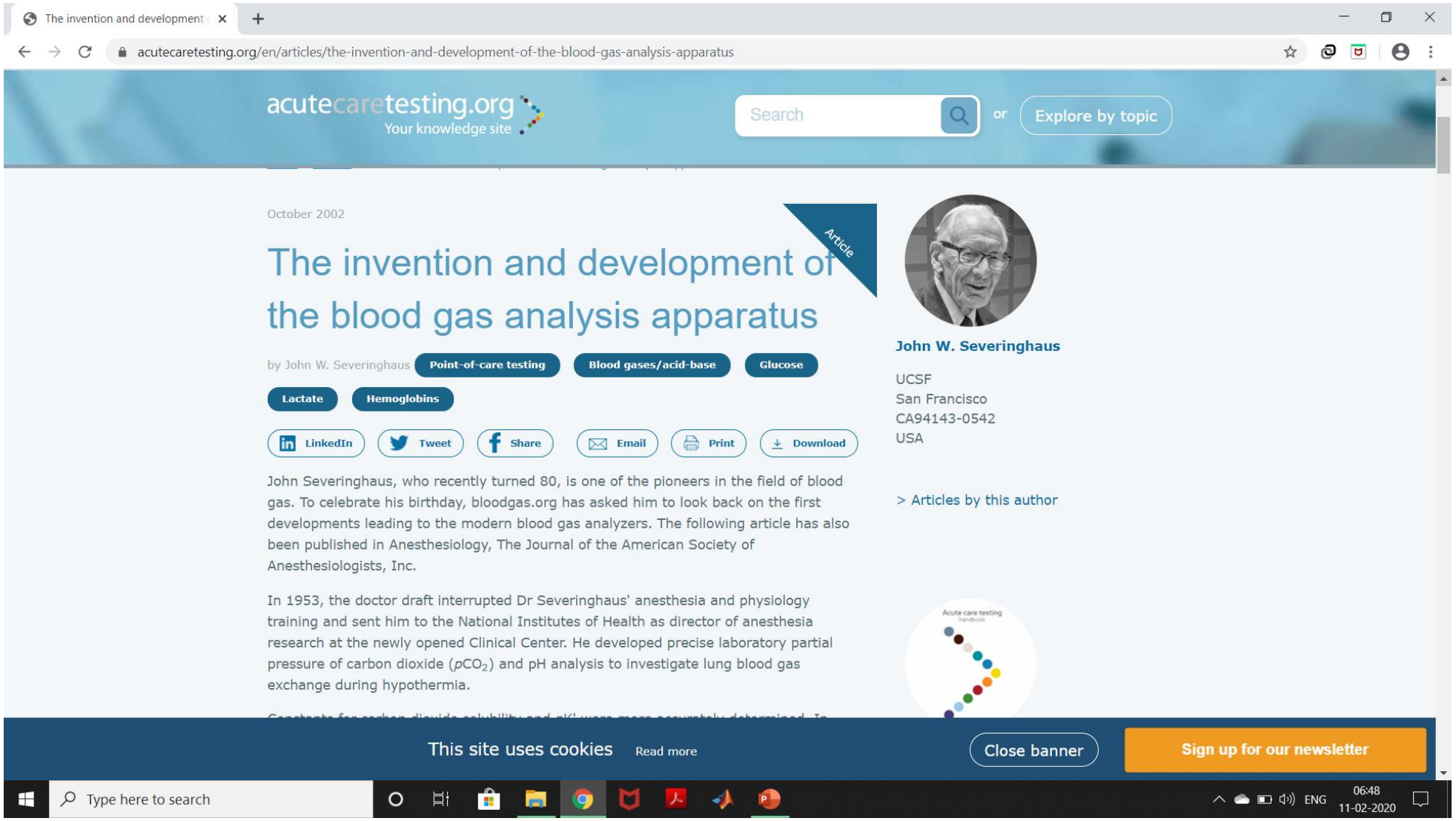
Analyzers used to measure blood gas, pH, electrolytes, and some metabolites in whole blood specimens. They can measure pH, partial pressure of carbon dioxide and oxygen, and concentrations of many ions (sodium, potassium, chloride, bicarbonate) and metabolites (calcium, magnesium, glucose, lactate). They are also used to determine abnormal metabolite and/or electrolyte levels in blood and the patient's acid-base balance and levels of oxygen/carbon dioxide exchange.

Product description

Handheld device or benchtop device, sometimes placed on a cart, with a display (usually LCD), a keypad to enter information, and a slot to insert a test strip or sample tube. Some models may have alarms, memory functions, touchpens, USB ports to transfer data to a computer, and/or a small storage compartment for reagents.

Use and maintenance

J W Severinghaus



J Clark The Founder of Oxygenator Electrode

Blood Cell Counters

Blood Particles

Changes in the normal functioning of an organism are often accompanied by changes in the blood cell count. Therefore, the determination of the number and size of blood cells per unit volume often provides valuable information for accurate diagnosis. The blood constitutes 5–10% of the total body weight and in the average adult, it amounts to 5–6 l. Blood consists of corpuscles suspended in a fluid called plasma in the proportion of 45 parts of corpuscles (cells) to 55 parts of plasma. The percentage of cells in the blood is called the haematocrit value or packed cell volume (PCV). The majority of the corpuscles in blood are red blood cells (erythrocytes), others being white blood cells (leucocytes) and platelets (thrombocytes).

Blood Cell Types

• **Table 16.1** ***Blood Cell Types***

Blood cell types	*Number of cells in* mm^3	*Mean cell volume (MCV) In* μm^3	*Relative proportion of different leucocyte count (differential)*
1. Erythrocytes	(4.8–5.5) $\pm 1 \times 10^4$	90	
2. Leucocytes	5000–10,000		100%
(a) Neutrophils	2000–7500	450	59 ± 18%
(b) Lymphocytes	1500–4000	250	34 ± 10%
(c) Eosinophils	40–400	450	2.5%
(d) Basophils	10–100	450	0.5%
(e) Monocytes	200–800	600	4%
3. Thrombocytes	$1.5 \times 10^5 - 4 \times 10^5$	8	–

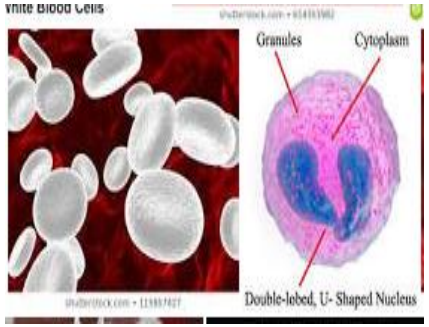
Erythrocytes (Red Blood Cells)

Erythrocytes (Red Blood Cells): Red blood cells have the form of a bi-concave disc with a mean

diameter of about 7.5 μm and thickness of about 1.7 μm. The mean surface area of the cell is about $134\mu m^{2.}$ There are about 5.5 million of them in every cubic milli metre of blood in men and nearly 5 million in women. In the whole body, there are about 25 billion erythrocytes and they are constantly being destroyed and replaced at a rate of about 9000 million per hour. The normal red cell lasts approximately 120 days before it is destroyed.

The erythrocytes have no nucleus. They are responsible for carrying oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. Anaemia (reduction in the oxygen carrying capacity of blood) can develop from a change in the number, volume or Hb concentration of erythrocytes, caused by bone marrow dysfunction resulting in the poor production rate of RBCs.

Since these changes are specific, the measurement of packed cell volume (PCV), the number of RBCs and the haemoglobin (Hb) are very important.


White Blood Cells
Granules
Cytoplasm
Double-lobed, U- Shaped Nucleus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White Blood Cells

Leucocytes (White Blood Cells): Leucocytes are spherical cells having a nucleus. There are normally 5000–10,000 white cells per cubic mm of blood but their number varies during the day. They live for seven to fourteen days and there is a rapid turn over, with constant destruction and replacement. Leucocytes form the defence mechanism of the body against infection. They are of two main types: the neutrophils and the lymphocytes. Neutrophils ingest bacteria/microbes responsible for infection. and **lymphocytes are concerned with immunological response, they respond to antigens and bind to them and generates antibodies**. The number and proportion of these types of leucocytes may vary widely in response to various disease conditions. For thus reason, it is important to know the total leucocyte count. The change, however, is often so small that the WBC count remains within normal limits and only the differential count would indicate any abnormality.

Neutrophils are nearly twice as big as the red cells and contain both a nucleus divided into several lobes and granules in their protoplasm. Lymphocytes are of the same size as the red cells but contain a large density staining nucleus and no granules. Monocytes are another type of leucocytes, which are twice as big as the neutrophils. They have a single large nucleus and no granules.

Thrombocytes (Platelets)

- ***Thrombocytes (Platelets):*** Platelets are usually tiny, round, oblong or irregularly shaped cells of the blood with an average diameter of approximately 2 μm. They play an important role in the blood coagulation process. There are usually 250,000–750,000 platelets in every cubic mm of blood.

Methods of Cell Counting

The most common and routinely applied method of counting blood cells even today, particularly in small laboratories, is the microscopic method in which the diluted sample is visually examined and the cells counted. Commonly known as the counting chamber technique, it suffers from several common drawbacks. Apart from the inherent manual error of the system, which may be about 10%, there is an additional subjective error of another problem with microscopic counting is that the data gathered by this measurement is not directly suitable for storage or for further processing and evaluation. Furthermore, the increasing number of examinations carried out in large series in busy laboratories necessitated the development of automatic instruments for counting the blood particles, with the errors of counting significantly reduced than a counting chamber. Agoston and Zillich (1971) compared the results of microscopic counting with those made by electronic counters (Fig. 16.1). The electronic counters provide a greater accuracy.

Counting Chamber is a microscopic slide to hold a known volume of the cell culture, between a grid and the cover. While counting the technician can move the grid.

Comparision of microscopic counting and electronic counting

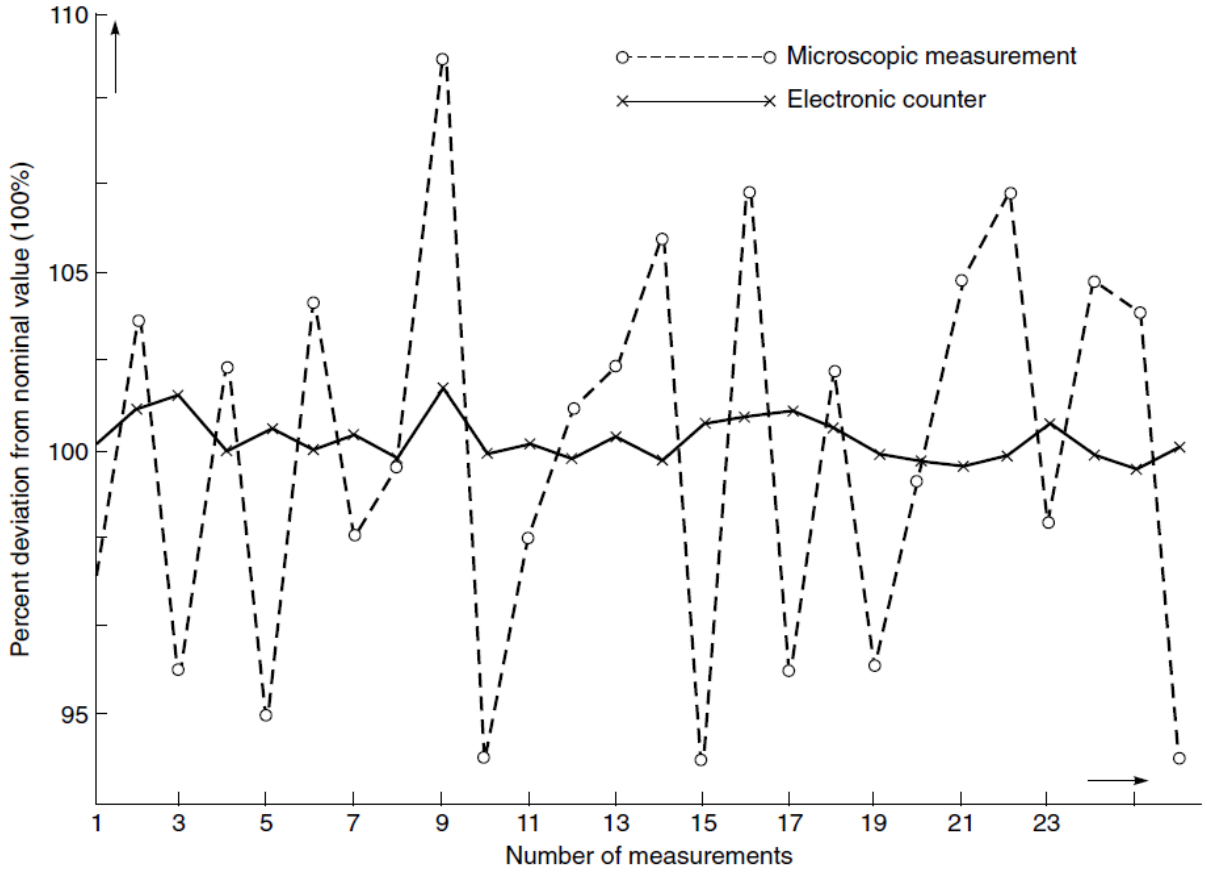
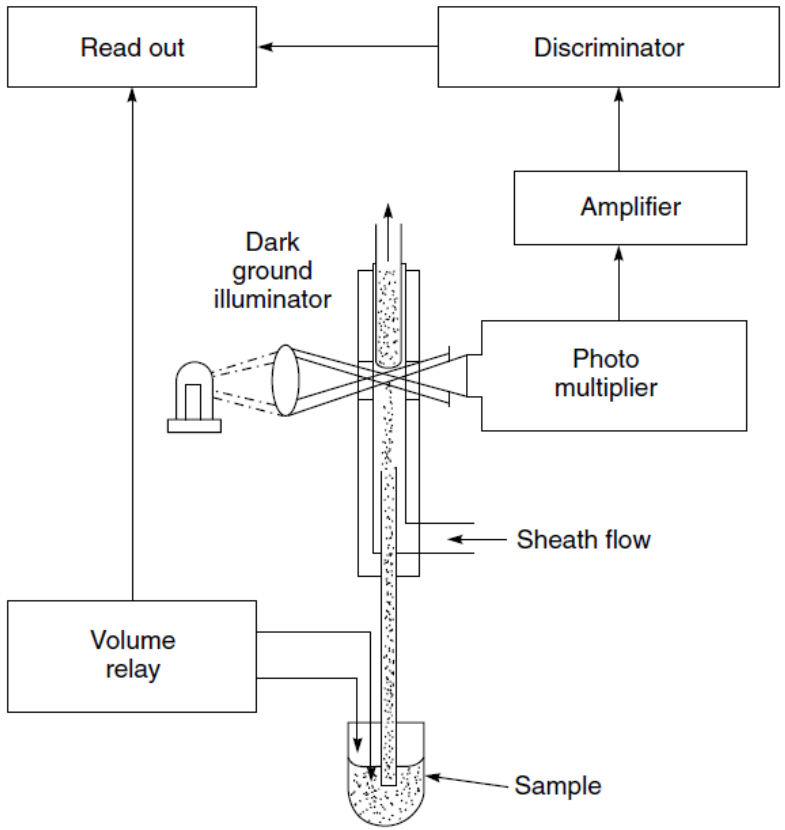


Fig. 16.1 *Comparison of results obtained with microscopic counting and*

Automatic Counting Method:
Optical Method

The method is based on collecting scattered light from the blood cells and converting it into electrical pulses for counting. Figure 16.2 shows one type of arrangement for the rapid counting of red and white cells using the optical detection system. A sample of dilute blood (1:500 for white cells and 1:50,000 for red cells) is taken in a glass container. It is drawn through a counting chamber in which the blood stream is reduced in cross-section by a concentric high velocity liquid sheath. This produces a stream of single particles blood stream. A sample optical system provides a dark field illuminated zone on the stream and the light scattered in the forward direction is collected on the cathode of a photomultiplier tube. Pulses are produced in the photomultiplier tube corresponding to each cell, as they absorb/scatter the light falling on them. Thus each particle results in a single pulse. The pulses counted for a fixed volume gives the exact count of the particle . These signals are amplified in a high input impedance amplifier and fed to an adjustable amplitude discriminator. The discriminator provides pulses of equal amplitude, which are used to drive a digital display. The relay connected to the sample holder ensures that a fixed small volume is drawn for measurement.

Instruments based on this technique take about 30 s for completing the count. An accuracy of 2% is attainable. The instruments require about 1 ml of blood sample.



➤ **Fig. 16.2** *Optical method of counting cells*

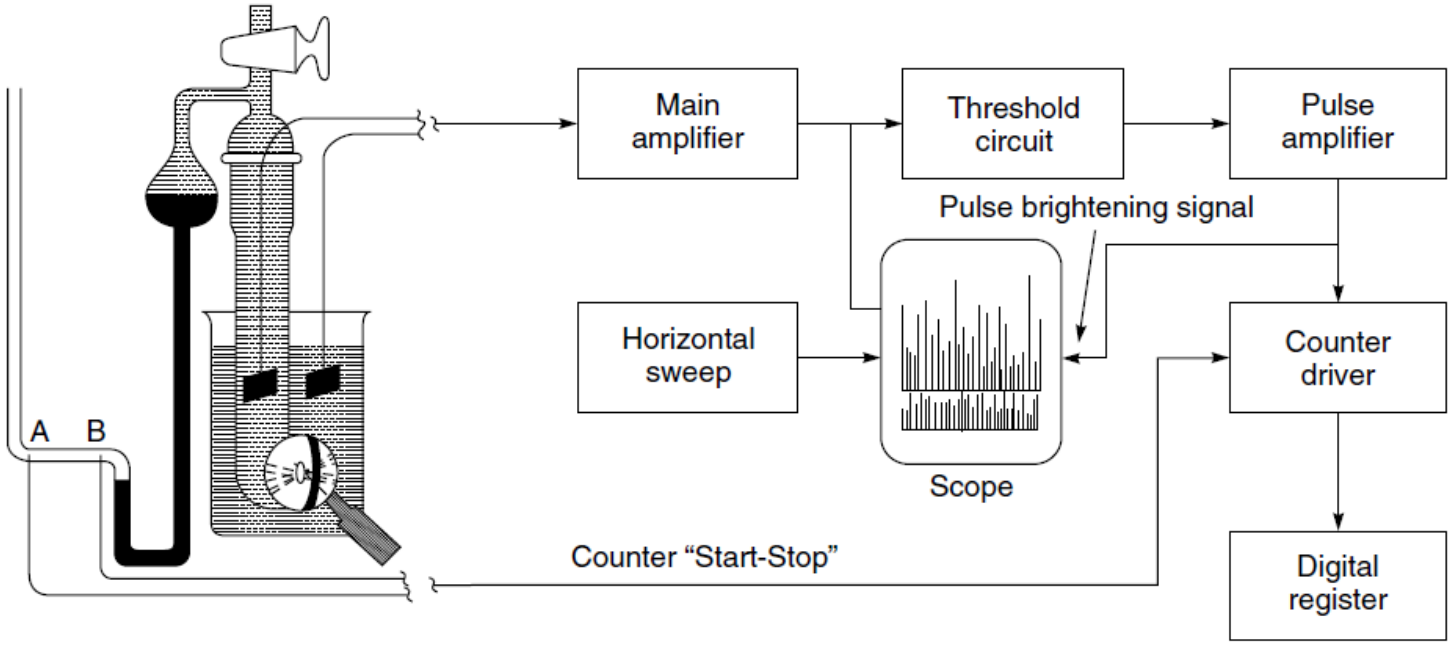
Coulter Counter

This manometer includes two platinum wire contacts (A and B) set through the glass walls. Contact A will start the count and contact B will stop it when precisely 0.5 ml of the dilution has passed through the orifice tube. Thus, it provides a count of the number of particles in a fixed volume of suspension. Figure 16.5 shows the sequence of building up the pulse in terms of increase in resistance at different positions of the cell with respect to the orifice. To enable the instrument to count only those pulses, which fall within certain preset size limits, the threshold facility is required. The threshold is also necessary to enable the instrument to ignore any electronic noise, which may be present in the system. The lower threshold sets an overall voltage level, which must be exceeded by a pulse before it can be counted. The upper threshold will not allow pulses to be counted which exceed its preset level.

Coulter Counter

A wide range of particle counting instruments designed to meet a wide variety of needs in the haemotology laboratory are being commercially produced. These instruments range from the small counters used primarily for red and white cell counts in very small hospitals and clinics, to the multi-parameter microprocessor controlled instrument featuring fully automatic diluting of samples and printing of results. Figure 16.4 shows a block diagram showing the principle of a Coulter counter. A platinum electrode is placed inside the orifice tube and a second electrode is submerged into the beaker containing the cell dilution, creating an electrical circuit between the two electrodes. Current will flow from one electrode to the other through the orifice. When the cell suspension is drawn through the orifice, cells will displace their own volume of electrolyte and cause a resistance change, which is converted to a voltage change, and is amplified and displayed. In practice, the cell suspension is drawn through the orifice by means of a mercury manometer.

Coulter Counter



➤ **Fig. 16.4** *Principle of Coulter counter*

The Coulter counters are usually provided with an oscilloscope monitor to display the pulse information, which has passed through the amplifier, and acts as a visible check on the counting process indicating instantaneously any malfunctions such as a blocked orifice. In particular, it provides information regarding (i) relative cell size, (ii) relative cell size distribution, (iii) settings of the threshold level control, and (iv) means to check the performance of the instrument for reliability of counts. The voltage pulses produced each time a cell passes through the orifice are displayed on the oscilloscope screen as a pattern of vertical spikes.

Coulter counters also help to give an idea of the size distribution of various types of cells. It has been stated that the pulse-height is to a first approximation, proportional to the volume of the particle. Converting the pulse height into a digital number, through an A-D converter, and storing it in memory can help to obtain a plot of the number of cells as a function of their size.

Calibration

The calibration factor is constant for a given aperture size, electrolyte resistivity and amplifier gain setting. It is used for the conversion of threshold settings to particle volumes or their cube roots to equivalent spherical diameters. Calibration is done simply and quickly by observing the threshold for monosized particles of known diameter (adjusting the threshold level to the peaks of the single-height pulses on the oscilloscope screen). Ragweed pollen (19 micron in diameter) and polystyrene latex particles (6–14 micron in diameter) seem to meet these requirements. Of the two, polystyrene latex is preferred for calibration purposes (Thom, 1972). The particles when used

seldom plug the orifice. These can be conveniently obtained in the range of 5 million particles per cubic mm.