Chapter II.10 p_{O_2} and Oxygen Content Measurement in Blood Samples Using Polarographic Oxygen Sensors

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1 Introduction

A major function of blood in most organisms is to supply the tissues with oxygen. The measurement of oxygen partial pressure (p_{O_2}) and oxygen content (c_{O_2}) in blood are therefore important criteria through which the state of the gas exchange system of the organism can be assessed.

Baumberger [2] initially used a polarographic technique to measure p_{O_2} in blood. Further improvements, notably the construction of the membrane-covered oxygen sensor [7], led to rapid advances, and today the measurement of p_{O_2} in blood is routinely performed with a polarographic oxygen sensor (POS) in most medical laboratories. The increasing importance of accurate blood p_{O_2} measurements in research laboratories either for the construction of dissociation curves [9, 29] or the measurement of oxygen content [6, 19, 22] has given more emphasis to the critical assessment of p_{O_2} measurement in blood. Several reviews are available on methodology and techniques used in human blood p_{O_2} measurements at 37°C [4, 12, 15, 19, 30–32]. However, the ecophysiologist working with nonmammalian vertebrates or invertebrates may encounter problems not experienced with human blood, e.g., nucleated red blood cells, different respiratory pigments, lower temperatures, limited availability of blood. The present chapter therefore attempts to review available information and provide some guide lines for p_{O_2} measurement for those who have ecophysiological interests.

2 p_{O_2} Measurement in Blood Using a POS

2.1 Materials and Apparatus Design

2.1.1 Polarographic Oxygen Sensor

A number of reviews are available concerning the performance of different commercial sensors in the laboratory [11, 15]. When measuring p_{O_2} in blood, the sample size is

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Polarographic Oxygen Sensors (ed. by Gnaiger/Forstner)

usually small and therefore a small cathode diameter (0.012–0.020 mm) is preferable. This is partly due to the reduction of the stirring effect and also due to the lower oxygen consumption of the small cathode. A 2 mm cathode has an oxygen consumption of 90 Pa min^{-1} in 0.1 cm^3 H_2O compared with 0.2 Pa min^{-1} for a 0.020 mm cathode [30].

The membrane is important in controlling the sensitivity and response time of the POS (Chap. I.1). A Teflon membrane is approximately 60 times as permeable to oxygen as a polypropylene membrane of similar thickness and therefore Teflon has a shorter response time τ_{99} = 10 s at 25 μm and 37°C compared with 25 s for a polypropylene membrane, [30]). Teflon membranes may exhibit greater gas/blood differences in p_{O_2} readings [21] and may undergo sensitivity changes with time due to their flexibility [15]. Polypropylene has a higher temperature coefficient for the response time. When working at temperatures of 15°C and lower, Teflon gives a much better response time. At higher temperatures, around 37°C, polypropylene may provide an adequate response time. Membranes should ideally be fitted to the sensor 24 h in advance of measurements and then they usually remain stable for at least an 8-day period, depending upon the amount of use [15].

Hahn et al. [13] have shown that the use of alkaline electrolytes (pH = 11.2) and a polarizing voltage of 0.9 V gives a marked improvement in both stability and response time. Details for an electrolyte giving a pH of 11.2 at 37°C are Na_2HPO_4 = 0.0587 g; KCL = 0.0745 g; and NaOH = 0.170 g in 100 cm^3 bi-distilled water.

Oeseburg et al. [24] have confirmed Hahn's findings and recommend the use of bicarbonate instead of phosphate buffer to add to stability (electrolyte = $NaHCO_3$ 0.08 mol cm^{-3}, KCL 0.1 mol dm^{-3}; NaOH added until pH = 11.2). They also suggest that the membrane holder and electrolyte chamber should be degassed after filling to improve stability. For further information see Chapter I.5.

2.1.2 Experimental Setup

Figure 1 shows an experimental setup (after N. Heisler and W. Nüsse) for replicate determination of p_{O_2} in blood samples. It consists of two sensor cuvettes (Radiometer D616) made of stainless steel and glass which enclose two POS (Radiometer E5047). The cuvettes are mounted in a perspex chamber (volume 1.25 dm^3). A large volume is used to damp down any temperature fluctuations and the chamber is thermostatted by an external source. Calibration gases are fed into the system via needle valves and PVC tubing (4 mm inner diameter, 1.5 mm outer wall thickness). From here the gases are passed through the humidifier at a flow rate of 25 cm^3 min^{-1}, which ensures that the gases are fully saturated with water vapor at experimental temperature. The gas calibration line (polypropylene, 1 mm inner diameter, 0.5 mm wall thickness) can be connected to the sensors via the Luer-Lock injection port (3 mm inner diameter) through which the blood sample is introduced. The POS are mounted such that sequential filling requires 0.2 cm^3 of blood which can be withdrawn and used for other measurements.

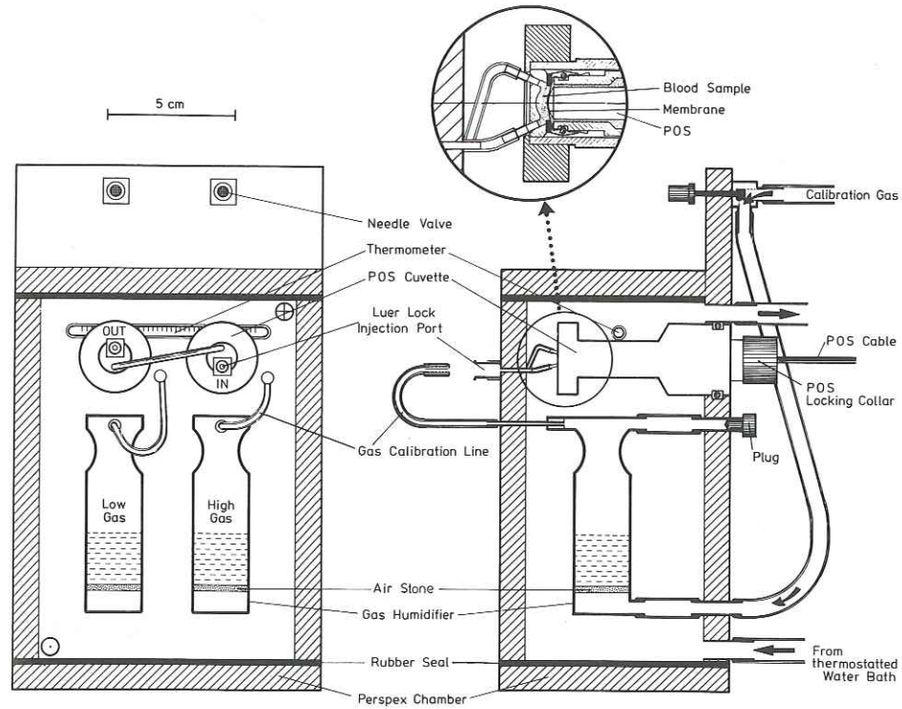

Fig. 1. p_{O_2} measurement system designed by N. Heisler and W. Nüsse, consisting of two POS mounted in a Perspex chamber, together with a calibrating gas humidification system

2.2 Calibration

Due to the fact that all commercially available sensors show deviation from linearity above 40 kPa it is suggested that, when working at p_{O_2} ranges above this value, a bracketing calibration is used [15]. When working in the normal physiological range for blood p_{O_2}, i.e., 1–13 kPa, calibration bracketing gives added accuracy but is not essential. Since the POS may show hysteresis or memory effects equilibration of the POS at the approximate p_{O_2} of the sample before injection is recommended. Moran et al. [21] have also suggested exposing the sensor to nitrogen before the calibration gas or blood sample. Alkaline electrolytes reduce the hysteresis effect [13].

Since the micro-cathode POS is designed to operate in unstirred solutions it has been found that liquid samples give lower p_{O_2} readings than their corresponding equilibrating gas [12, 30, 34]. Table 1 illustrates some gas/liquid factors measured at different temperatures with different membranes. Doubly distilled deionized water gives the closest approximation to the gas value and there is considerable variation between blood and water. Therefore when calibrating with water it is important to determine the water/blood factor by equilibrating the blood with the same gas as in the water calibration and measuring the p_{O_2} in both liquids. If gas calibration is used, the gas/blood factor must be ascertained. A number of parameters may influence the gas/blood

Table 1. Gas/blood factors

Sample	Temp. (°C)	Membrane [a]	p_{O_2} Range [kPa]	$\frac{p_{O_2}\text{ gas}}{p_{O_2}\text{ sample}}$	Ref.
Human blood	37	Polypro.	0–93	1.100	[21]
Human blood	37	Teflon	0–93	1.230	[21]
Human blood	37	Polypro.	0–93	1.044	[30]
50% Glycerine/H_2O	37	Polypro.	0–93	1.126	[30]
Chicken blood	40	Polypro.	0–87	1.145	[23]
Dog blood	40	Polypro.	0–87	1.057	[23]
1.0 M NaCl	40	Polypro.	0–87	1.035	[23]
H_2O [b]	40	Polypro.	0–87	1.003	[23]
Dogfish blood	15	Teflon	–	1.150	N. Heisler (pers.comm.)
Trout blood	15	Teflon	0–13	1.101	This study
Eel blood	15	Teflon	0–13	1.141	This study

Polypro. = Polypropylene

[a] All membranes approximately 25 μm thick
[b] Doubly distilled deionized water

factor, such as the type of membrane and its thickness, Teflon giving larger gas/blood factors than polypropylene [21]. Nightingale et al. [23] found that the gas/blood factor for bird blood increased with increasing hematocrit and therefore increasing viscosity. It is clear that each experimental setup with different blood will give different gas/blood factors. The gas/blood factor must therefore be measured for each experimental series on the same blood as used for the p_{O_2} measurements. This can be done by pooling the used blood samples and then tonometering the blood at known p_{O_2} values and measuring both tonometer gas and blood p_{O_2}.

2.3 Measurement

The following procedure is used when making measurements with the system as shown in Fig. 1. Whenever possible the instruments are allowed to warm up and the POS to adjust to the measuring temperature overnight. A calibration for the zero point is made by flowing oxygen-free nitrogen through the humidifier system and past the sensors in their cuvettes for 10 min. A high calibration gas at around 12 kPa p_{O_2} is then provided by a gas mixing pump (301 a-f Wösthoff, Bochum, West Germany) and the p_{O_2} reading adjusted. The linearity of the response is then checked with a low calibration gas supplied by the mixing pump at around 2.7 kPa p_{O_2}. Prior to the injection of the sample, the cuvettes are rinsed with physiological saline, to prevent any hemolysis of blood by water contact, and then either the high gas or low gas calibration is applied, depending on whether arterial or venous samples are to be measured. When the POS has come into equilibrium, the gas line is disconnected and the blood sample slowly injected, being careful not to generate pressure gradients or trap gas bubbles in the cuvettes. The response of the sensors can be followed on a calibrated chart recorder

(BD 9, Kipp & Zonen, Holland). When no recorder is available, a stopwatch can be used to make readings after 1, 1.5, 2, and 3 min. Depending on the response of a given blood (Fig. 2B–D) an extrapolation back to time zero can be made to determine the p_{O_2}. After a reading the sample is pushed out of the system and pooled for gas/blood factor measurement. The measured p_{O_2} is then multiplied by this factor to give the actual p_{O_2}. The cuvettes are flushed slowly until clean with a saline solution which has been equilibrated at the same temperature and p_{O_2} as the calibration gas. The measuring procedure is repeated for the next sample. After completion of all measurements the POS cuvette is filled with a bio-detergent (Radiometer enzyme electrode detergent S4160) and left overnight.

2.4 Problems and Sources of Error in p_{O_2} Measurements

2.4.1 Reproducibility

If measurements are made repeatedly on the same sample, rinsing with saline and calibrating with air in between, a coefficient of variation of around 2% is expected [32]. Gleichmann and Lübbers [12] reported a reproducibility of 0.5% for double determinations on blood up to 40 kPa and Moran et al. [21] found that repeated determinations on blood, equilibrated with gas tensions within the physiological range, gave a standard deviation of 1.7% and 1.2% for Teflon and polypropylene membranes respectively. Using the system shown in Fig. 1 Scheid and Meyer [29] found in experiments on human, rabbit and duck blood that the difference in p_{O_2} reading of the first and second POS, filled sequentially from a syringe, for duplicate determinations, averaged 0.01 ± 0.05 (SD) kPa for 165 measurements.

2.4.2 Temperature

Temperature control to within ± 0.1°C is of critical importance. Human blood, and probably blood of other animals as well, has a high temperature coefficient and a 1°C change in temperature will change the blood p_{O_2} by 6%–7% [31]. The temperature change will effect the sensor response itself giving a total error of 10%. Measurements should be carried out at the in vivo temperature of the blood for the greatest accuracy. Where this is not possible then a series of nomograms is available for human blood [14, 16] which, with some reservations, can be applied to temperature corrections for other bloods.

2.4.3 Blood Metabolism

Mammalian blood has non-nucleated red blood cells and a relatively low oxygen consumption. The fall in p_{O_2} of stored blood samples is dependent on the temperature and the initial p_{O_2} [10, 26]. At 37°C and normal physiological p_{O_2} values the fall in p_{O_2} is less than 0.07 kPa min^{-1}, compared with 0.3–0.4 kPa min^{-1} at p_{O_2} values above 40 kPa. Cooling of the sample to around 0°C reduced the p_{O_2} decline to 0.4 kPa

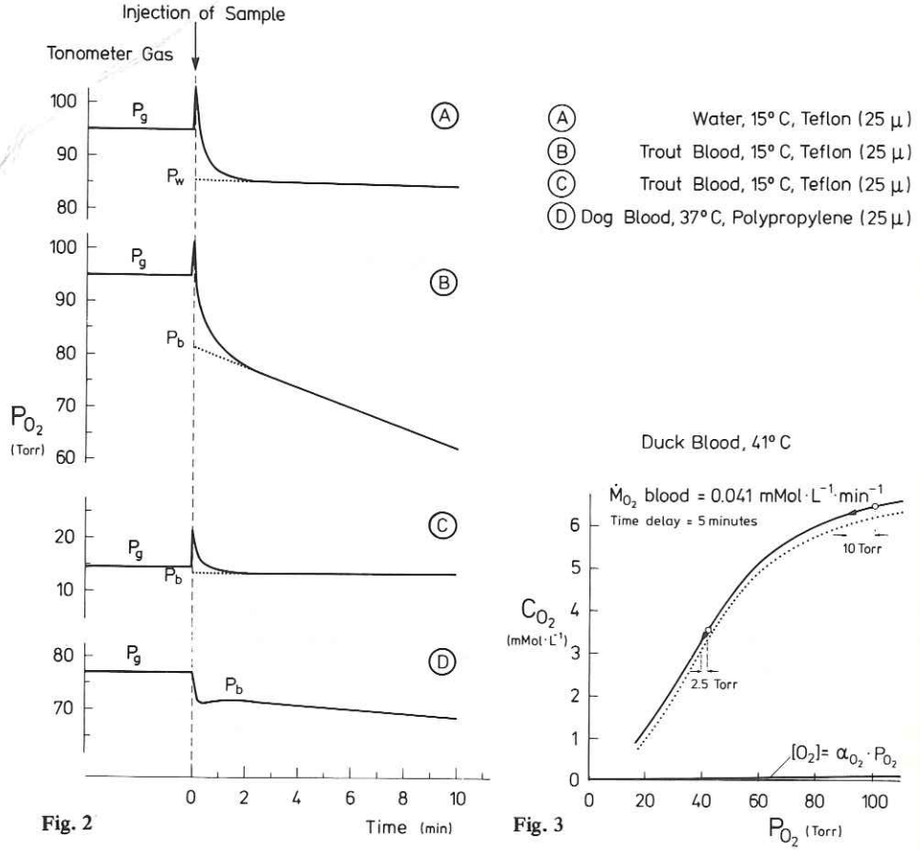

Fig. 2

Fig. 3

Fig. 2. Actual recordings of p_{O_2} measurements made with a POS for various liquids and different equilibrating gas levels. p_g = partial pressure of oxygen in tonometer gas; p_w = partial pressure of oxygen in water; p_b = partial pressure of oxygen in blood; p_w and p_b = "instrumental" p_{O_2}'s and have to be corrected by the gas/liquid factor (see text)

Fig. 3. Oxygen dissociation curve for duck blood (modified after [28]) shows the effect of blood metabolism on the measurement of p_{O_2}. *Continuous line* is the true dissociation curve. Time delay of 5 min results in a down shift in curve by the amount of oxygen which is metabolized (*broken curve*). *Arrows* indicate drop of c_{O_2} due to metabolism and thus p_{O_2} change during storage

min^{-1} even at high p_{O_2} values. The problem of blood metabolism is more severe when working with nucleated red blood cells as found in nonmammalian vertebrates and some annelids. Here metabolism may be several times higher than that of human blood even at lower temperatures. Figure 2B–D illustrates some case studies of p_{O_2} measurement in blood. When water is equilibrated with tonometer gas (p_g) at around 13 kPa (= 95 mm Hg) and injected into the cuvette system (as in Fig. 1), the sensor gives a relatively stable reading for the water p_{O_2} (p_w) after 2 min with negligible oxygen consumption thereafter (Fig. 2A). If trout blood is equilibrated at the same gas tension, which approximates to arterial values, and injected into the cuvette, then a rapid

fall in p_{O_2} is seen (Fig. 2B) until after 2 min, a steady decline in p_{O_2} is given at approximately 0.27 kPa min^{-1} (= 2 mm Hg min^{-1}) which is mainly due to blood metabolism. Therefore to determine the accurate p_{O_2} of the blood (p_b), an extrapolation back to time zero is made. If the blood is equilibrated at low p_{O_2} values (2 kPa) which approximate to venous blood, then a similar response to that shown by water is seen (Fig. 2C) with a steady reading after 2 min followed by a very slow decline. Extrapolation back to zero in this case only gives a change in p_b of 0.07 kPa (= 0.5 mm Hg) compared with 0.5 kPa (= 4 mm Hg) at high p_b values. In comparison, when dog blood, which has no nucleated red cells, is measured at arterial values a plateau is given between the first and second minute after injection (Fig. 2D) and this value is generally taken as p_b.

These differences in the rate of change of p_{O_2} between high and low p_{O_2} values for blood in general can be explained by the shape of the oxygen dissociation curve for blood. As an example Fig. 3 shows the oxygen dissociation curve for duck blood [28]. Avian blood has a very high metabolism, up to ten times that of human blood, and is similar to fish blood at the same temperature. Scheid and Kawashiro [28] found that the oxygen content of a blood sample would decrease by 0.2 mmol dm^{-3} during a 5-min delay in measurement. At high p_{O_2} values around 13 kPa (= 100 mm Hg), where the dissociation curve flattens off as the hemoglobin approaches 100% saturation (Fig. 3), a fall in oxygen content of 0.2 mmol dm^{-3} gives a 1.3 kPa (= 10 mm Hg) change in p_{O_2}. For the same oxygen content change at around the P_{50} only a fall of 2.5 mm Hg is given due to the steepness of the dissociation curve. It is therefore evident that when making p_{O_2} measurements in metabolically active blood at p_{O_2} ranges where the respiratory pigment approaches 100% saturation, some knowledge of the rate of metabolism is necessary in order to avoid errors in the measurement. Scheid and Kawashiro [28] calculated that for duck blood at 20 kPa a drop of p_{O_2} of 4 kPa min^{-1} could be expected with virtually all of the oxygen consumption being satisfied by physically dissolved oxygen. To avoid metabolic effects in blood samples arterial blood should, whenever possible, be measured immediately. Failing this, storage on ice, together with venous samples, whose rate of p_{O_2} change with time is small, is recommended.

2.4.4 Calibration and Tonometry

If borate/sulfite solutions are used for zero calibration, they must be fully rinsed from the system as even small residual amounts can cause depletion of p_{O_2} in blood samples. Ideally for liquid calibration equilibrated blood should be used, but this is not practical when available blood volumes are small. Therefore when determining either water/blood factors (p_w/p_b) or gas/blood factors (p_g/p_b), as in Fig. 2B–C, with metabolically active blood care must be taken to ensure complete equilibration between gas and blood in the tonometer. Thin film tonometers should be used and the equilibrating gas should contain a carbon dioxide tension similar to that found in vivo to ensure that the blood remains within the range of the normal dissociation curve. When working at low temperatures, equilibration times should be adjusted accordingly to ensure complete equilibration. Sampling syringes should be carefully rinsed with

tonometer gas before taking up the sample, and transfer to the POS accomplished in as short a time as possible, avoiding large temperature gradients. Gas/blood factors should be ascertained at both arterial and venous p_{O_2} ranges as these may differ [11, 26]. The use of solutions with similar physical properties to blood such as glycerine/water mixtures has been advocated [15, 30] to overcome tonometry problems. However, these provide only a partial answer since the gas/blood factor varies with viscosity and thus differs with hematocrit and different respiratory pigments. Therefore, blood should be used to determine the correction factors.

2.5 Summary

The following is a summary of guidelines for p_{O_2} measurement in blood:

1. Cathode diameter, membrane, electrolyte and polarizing voltage are selected to give a fast and stable response at the selected measuring temperature, which must be maintained to within ± 0.1°C.
2. For liquid calibration borate/sulfite solutions and air-equilibrated water are used to provide zero p_{O_2} and the high calibration point respectively.
3. Calibration with humidified gases should be used whenever possible, checking linearity of response by calibration bracketing.
4. The POS must be equilibrated at a p_{O_2} similar to that of the sample before each measurement.
5. The response of the POS should be followed and a consistent measuring procedure determined for a given blood.
6. Water/blood or gas/blood correction factors should be determined for each measurement series.
7. To avoid errors due to metabolism, arterial samples should be measured immediately or else stored on ice.
8. The metabolic activity of the blood should be determined when working with nucleated red blood cells at high p_{O_2} ranges.

3 Oxygen Content Measurement Using a POS

3.1 Theory

Consider a volume, V [cm^3], of a solution which displaces chemically bound oxygen from a respiratory pigment. The initial partial pressure of oxygen in this solution, p_s [kPa], can be determined with a POS. A small volume of blood (v) is injected into the solution such that it displaces a corresponding amount of the solution from the containing vessel which is immediately closed. The oxygen content of the solution, c_s [μmol O_2], can be determined if the solubility of oxygen in the solution, S_s [μmol cm^{-3} kPa^{-1}] (App. A), is known.

$$c_s = p_s \times S_s \times (V - v). \tag{1}$$

When the blood and solution are mixed, chemically bound oxygen is released from the pigment and since the system is a closed one then the chemically bound oxygen is converted into physically dissolved oxygen and the oxygen partial pressure in the system rises to a new level (p_{s+b}). The oxygen content of the mixture will now consist of that of the solution (c_s) and that of the blood (c_b) such that

$$c_s + c_b = p_{s+b} \times S_s \times V. \tag{2}$$

Since the ratio V/v is usually large, then the added blood will not significantly affect the solubility of oxygen in the solution. The total oxygen content of the blood, c_b [μmol O_2 cm^{-3} blood] can be calculated from Eq. (2) such that

$$c_b = S_s \times \frac{V}{v} \times \left(p_{s+b} - \frac{V - v}{V} \times p_s\right). \tag{3}$$

S_s, V and v are known and p_s and p_{s+b} are measured using a POS, therefore total oxygen content of the blood can be determined.

3.2 Materials and Apparatus

3.2.1 Displacement of Chemically Bound Oxygen

Previous manometric methods for oxygen content measurement [27, 38] utilized chemical agents to release oxygen bound to respiratory pigments and these can be utilized for oxygen content measurement with the POS. Table 2 illustrates the use of different reagents for various respiratory pigments. Potassium ferricyanide solutions (6 g dm^{-3}) have generally been used for hemoglobin oxygen content determinations, with saponin added (3 g dm^{-3}) to aid cell lysis. Due to the instability of saponin, it is recommended to make up the solution freshly every day and store it in a dark bottle. The use of carbon monoxide-saturated saline has been preferred in some measurements where small bore stopcocks are used, as it does not lyse cells and thus prevents the accumulation of cell debris in the apparatus [17]. The addition of a small quantity of an anti-foaming agent such as caprylic alcohol to the solution is also recommended. Rawlinson [25] has suggested that potassium cyanomercurate be used instead of potassium cyanide to avoid oxygen reabsorption problems, but Truchot [36] found no significant difference in oxygen content determinations on hemocyanin using both cyanide and cyanomercurate.

Table 2. Chemical reagents for the release of bound oxygen from respiratory pigments

Respiratory pigment	Reagent	Ref.
Hemoglobin	Potassium ferricyanide K_3 [$Fe(CN)_6$]	[8, 18, 22, 37, 38]
	Carbon monoxide CO	[1, 3, 17]
Hemocyanin	Potassium cyanide KCN	[6, 8, 36]
	Potassium cyanomercurate K_2 [$Hg(CN)_4$]	[25]
Hemerythrin	Potassium ferricyanide	[8, 39]

3.2.2 *Apparatus*

Neville [22] employed a simple mixing vial for blood and ferricyanide and measured the increase in p_{O_2} with a mercury dropping electrode. A simple and accurate mixing system can be constructed out of graduated syringes and a three-way tap, utilizing a glass or mercury bead to facilitate mixing, and then measuring p_{O_2} with a separate POS [18, 35].

Mayers and Forster [20] utilized the bores of two stopcocks to give precise mixing volumes of blood and ferricyanide. The method was modified [17] using a five-port, double-bore stopcock, replacing ferricyanide with carbon monoxide equilibrated saline and using a volume limited syringe to assure reproducibility of dilution volumes.

A number of chamber designs in perspex, glass, and stainless steel are available which incorporate the POS in the mixing chamber and thus avoid excessive handling of the samples [1, 5, 6, 33, 37]. Since the change in oxygen tension (Δp_{O_2}), due to the release of chemically bound oxygen from the pigment, is proportional to $c_b \times \nu/V \times S_s^{-1}$, then the chamber volume (V) and injection volume (ν) are selected such that Δp_{O_2} is large for a given blood oxygen content c_b. When working with hemoglobins which have a relatively high oxygen content even at low hemoglobin saturations compared with hemocyanins, chamber volumes of 2.2–2.55 cm^3 and injection volumes of 22–72 mm^3 have been used [1, 5]. Tucker [37] using a small chamber volume (0.5 cm^3) and a 7 mm^3 injection volume obtained a $\Delta p_{O_2} > 13$ kPa for fully saturated human hemoglobin. With hemocyanin even at full saturation, oxygen content is usually below 5 vol-% and therefore a small chamber volume is preferable especially if the available blood volume is limited.

Figure 4 illustrates a chamber constructed out of perspex [6] for the measurement of oxygen content in blood containing hemocyanin. It consists of a POS (Radiometer E5047) with the outer sleeve removed and a special perspex sleeve constructed which allows the O-ring, which normally secures the membrane at the tip of the POS, to also

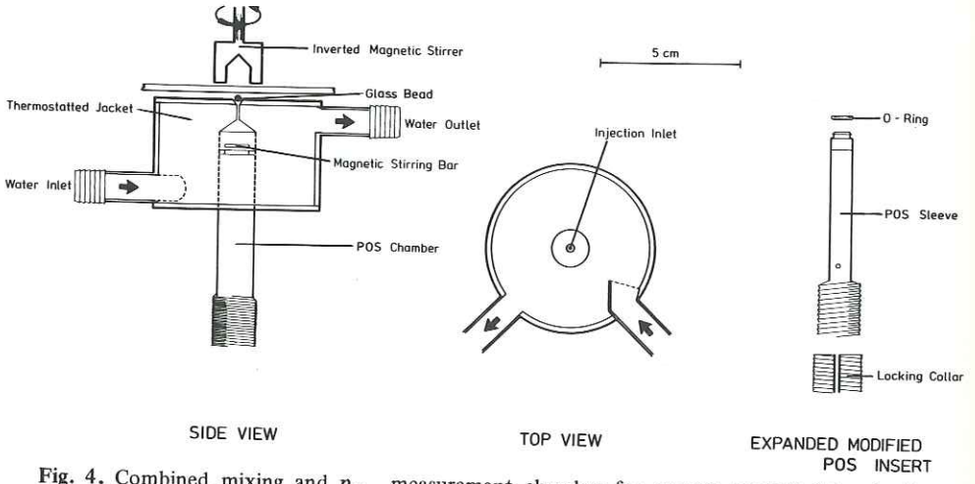

Fig. 4. Combined mixing and p_{O_2} measurement chamber for oxygen content determinations (after [6])

form a watertight seal with the floor of the sample chamber (0.5 cm^3). A magnetic stirring bar is enclosed within the chamber and is driven by an external inverted magnetic stirrer (Cenco, Neth.) placed on top of the chamber. The chamber can be sealed with a small glass bead which is dropped into a concave depression at the top of the injection inlet (1 mm diameter) after the injection of the blood sample. The roof of the sample chamber is cone-shaped to aid in the removal of air bubbles trapped in the chamber. Chamber and POS are thermostatted at ± 0.1°C of the selected temperature by a water jacket which is supplied by an external source.

3.3 Measurement

For measurements of oxygen content in blood containing hemocyanin, a temperature of 32°C was selected for the POS and the sample chamber as this temperature facilitates the displacement of chemically bound oxygen from the respiratory pigment. The sample chamber is first filled with a zero p_{O_2} solution (1 mg Na_2SO_3 + 5 ml 0.01 M $Na_2B_4O_4$ solution) and the zero point set. The solution is then aspirated from the chamber using a shortened hypodermic needle with a flattened tip. A needle tip diameter of 0.8 mm (21G) is used which allows air to enter the chamber as the fluid is aspirated from the chamber. The sample chamber is rinsed with distilled water to remove all traces of sulfite and then filled with an air equilibrated solution of potassium cyanide (6 g dm^{-3}) which is maintained in a water bath at the same temperature as the POS and the sample chamber. A small air bubble is left in the sample chamber to maintain air equilibration and the magnetic stirrer switched on. This second calibration point is set and the calibration solution aspirated from the chamber. A 20-cm^3 syringe fitted with a three-way tap is used to draw up 15 cm^3 of the KCN stock solution from the water bath. The oxygen tension of the cyanide solution is lowered by closing the tap and pulling on the syringe plunger, causing a decrease in pressure in the syringe. Dissolved gases come out of solution and the gas bubbles are expelled via the three-way tap. This operation is repeated a number of times until the solution p_{O_2} is low ($p_{O_2} < 5$ kPa). Alternatively, the KCN solution can be bubbled with nitrogen in the water bath if this is convenient.

The sample chamber is now filled with the low p_{O_2} KCN, the stirrer bar switched on and after equilibration (usually 30 s) a steady recording is obtained (p_s). A 100-mm^3 syringe (Hamilton 1710), fitted with a Chaney-adaptor for reproducibility, is used to inject a 65 mm^3 sample of blood into the chamber. A long blunt-ended needle (21G) is used to introduce the sample near the bottom of the chamber displacing an equivalent amount of KCN solution from the chamber. The needle is withdrawn and the injection port closed with a glass bead and the stirrer switched on. The response of the POS is followed, as previously, with a recorder until a stable reading is obtained (p_{s+b}). After the measurement the blood/KCN mixture is aspirated from the chamber and either the aerated calibration solution or the low p_{O_2} solution used to prepare for the next measurement. Each determination takes approximately 5 min to complete. The volume of the chamber and the injection volume can be found by weighing before and after filling them with distilled water. Values for S_s can be found in Tucker [37] or

Laver et al. [19] for a given chamber temperature. Equation (3) is then used to calculate the total oxygen content of the blood.

3.4 Reproducibility, Accuracy and Problems

Previous manometric methods for oxygen content determinations have the disadvantage of being time-consuming and generally require skilful manipulation of the apparatus. The measurement of oxygen content using a POS is relatively rapid and simple, requiring no special skills. Table 3 illustrates values for reproducibility of the various POS methods and their accuracy compared with the Van Slyke determination. Reproducibility is relatively good, being within ± 0.3 vol-% for all methods, which compares favorably with similar Van Slyke determinations [17, 18, 20]. Accuracy varies with the different methods but can be high [37]. Both accuracy and reproducibility will depend upon oxygen tension measurements and are therefore open to similar errors as mentioned previously for blood p_{O_2} measurements. Ideally Δp_{O_2} should be kept large to avoid errors and also to ensure that the injection volume is known accurately. A weighing procedure similar to that suggested by Scheid and Meyer [28] for O_2 equilibrium curves could be adopted to improve accuracy. Since calibration and measurements are made in the same liquid, gas/blood factors are not required. The use of the combined mixing chamber and POS, with a stirring bar, prevents the formation of diffusion gradients in the sample. However, problems may arise when working with metabolically active bloods, especially when there is a large difference between in vivo blood temperature and that of the mixing chamber and POS, as experienced with fish blood. Again careful assessment of the POS response is necessary. Errors due to the change in solubility of the solution after the addition of blood are very small and Laver et al. [18] could find no significant difference in solubility between ferricyanide solutions and blood/ferricyanide mixtures. Since the solutions themselves are very

Table 3. Reproducibility and accuracy of oxygen content measurements using a POS

Method	Blood volume [mm^3]	Reproducibility [vol-%][a]	Accuracy [vol-%][b]	Ref.
Mixing vial and separate POS	250	± 1.0% R	± 0.40	[22]
Syringe mixing and separate POS	50	0.22 ± 0.3 D	0.42 ± 0.54	[18]
Syringe mixing and separate POS	5	0.21 ± 0.13 D	0.16 ± 0.14	[35]
Syringe mixing and separate POS	10	0.16 ± 0.13 D	0.16 ± 0.11	[35]
Stopcock bore and separate POS	120	2% ± 0.29 D	0.37 ± 0.50	[20]
Stopcock bore and separate POS	100–200	± 0.07 D	0.54 ± 0.49	[17]
Mixing chamber and POS combined	72	± 0.18 R	–	[1]
Mixing chamber and POS combined	7	± 0.14 R	0.03 ± 0.17	[37]
Mixing chamber and POS combined	150–1000	0.14 ± 0.11 R	0.20 ± 0.16	[33]
Mixing chamber and POS combined	25	± 0.25 R	–	[5]
Mixing chamber and POS combined	65	± 0.064 R	–	[6]

[a] Reproducibility expressed as the mean difference between duplicates (D) or replicates (R) ± S.D.
[b] Accuracy expressed as the mean difference between a Van Slyke determination and POS method ± S.D.

dilute, then there is little difference in their solubility compared with water and for a 2 g dm^{-3} solution solubility changes by less than 0.5% [18]. Care must be taken to maintain a large V/v ratio, for if this becomes too small then the blood will affect the solubility of the solution and sufficient reagent may not be present to release all the chemically bound oxygen from the pigment. The addition of 0.01 g of ferricyanide to 2 cm^3 of blood is sufficient to liberate all the oxygen from oxyhemoglobin [38]. Therefore for a chamber volume of 0.5 cm^3 and a 6 g dm^{-3} solution of ferricyanide sufficient reagent is present for up to 0.6 cm^3 of blood.

3.5 Summary

1. Select reagent for displacement of chemically bound oxygen, according to respiratory pigment and ease of use.
2. Select chamber or syringe volume and injection volume to give a large Δp_{O_2} for a given oxygen content range.
3. Calibrate POS with (a) zero p_{O_2} solution, (b) aerated reagent solution.
4. Lower initial p_{O_2} of reagent, such that Δp_{O_2} is within the calibrated range of the POS.
5. Measure initial p_{O_2} of the reagent, inject known blood volume and measure p_{O_2} of the mixture, monitoring the response of the POS.
6. Total oxygen content of the blood is then determined from the known values of chamber or syringe volume, injection volume and the solubility of oxygen in the reagent, together with the measured p_{O_2} values.

References

1. Awad O, Winzler RT (1961) Electrochemical determination of the oxygen content of blood. J Lab Clin Med 58:489–494
2. Baumberger JP (1938) Determination of the oxygen dissociation curve of oxyhaemoglobin by a new method. Am J Physiol 123:10
3. Baumberger JP (1940) The accurate determination of haemoglobin oxyhaemoglobin and carbon monoxide haemoglobin (or myoglobin) by means of a dropping mercury electrode. Am J Physiol 129:308
4. Bishop JM, Pincock AC, Hollyhock A, Raine J, Cole RB (1966) Factors affecting the measurement of the partial pressure of oxygen in blood using a covered electrode system. Respir Physiol 1:225–237
5. Borgström L, Hägerdal M, Lewis L, Pontén U (1974) Polarographic determination of total oxygen content in small blood samples. Scand J Clin Lab Invest 34:375–380
6. Bridges CR, Bicudo JEPW, Lykkeboe G (1979) Oxygen content measurement in blood containing haemocyanin. Comp Biochem Physiol 62A:457–462
7. Clark LC Jr (1956) Monitor and control of blood and tissue oxygen tensions. Trans Am Soc Artif Int Organs 2:41–48
8. Cook SF (1927) The action of potassium cyanide and potassium ferricyanide on certain respiratory pigments. J Gen Physiol 11:339–348
9. Duvelleroy MA, Buckles RG, Rosenkaimer S, Tung C, Laver MB (1970) An oxyhemoglobin dissociation analyser. J Appl Physiol 28:227–233

10. Eldridge F, Fretwell LK (1965) Change in oxygen tension of shed blood at various temperatures. J Appl Physiol 20:790–792
11. Flenley DC, Millar JS, Rees HA (1967) Accuracy of oxygen and carbon dioxide electrodes. Br Med J 2:349–352
12. Gleichmann U, Lübbers DW (1960) Die Messung des Sauerstoffdruckes in Gasen und Flüssigkeiten mit der Pt-Elektrode unter besonderer Berücksichtigung der Messung im Blut. Pfluegers Arch Ges Physiol 271:431–455
13. Hahn CEW, Davis AH, Albery WJ (1975) Electrochemical improvement of the performance of p_{O_2} electrodes. Respir Physiol 25:109–133
14. Hedley-Whyte J, Radford EP Jr, Laver MB (1965) Nomogram for temperature correction or electrode calibration during p_{O_2} measurements. J Appl Physiol 20:785–786
15. Heitmann H, Buckles RG, Laver MB (1967) Blood p_{O_2} measurements: Performance of microelectrodes. Respir Physiol 3:380–395
16. Kelman G, Nunn JF (1966) Nomograms for correction of blood p_{O_2}, p_{CO_2}, pH and base excess for time and temperature. J Appl Physiol 21:1484–1490
17. Klingenmaier CH, Behar MG, Smith TH (1969) Blood oxygen content measured by oxygen tension after release by carbon monoxide. J Appl Physiol 26:653–655
18. Laver MB, Murphy AJ, Seifen A, Radford ER Jr (1965) Blood O_2 content measurements using the oxygen electrode. J Appl Physiol 20:1063–1069
19. Laver MB, Seifen A (1965) Measurement of blood oxygen tension in anesthesia. Anesthesiology 26:73–101
20. Mayers LB, Forster RE (1966) A rapid method for measuring blood oxygen content utilizing the oxygen electrode. J Appl Physiol 21:1393–1396
21. Moran F, Kettel LJ, Cugell DW (1966) Measurement of blood p_{O_2} with the macrocathode electrode. J Appl Physiol 21:725–728
22. Neville JR (1960) A simple, rapid polarographic method for blood oxygen content determination. J Appl Physiol 15:717–722
23. Nightingale TE, Boster RA, Fedde MR (1968) Use of the oxygen electrode in recording p_{O_2} in avian blood. J Appl Physiol 25:371–375
24. Oeseburg B, Kwant G, Schut JK, Zijlstra WG (1979) Measuring oxygen tension in biological systems by means of Clark-type polarographic electrodes. Proc K Ned Akad Wet Ser C 82:83–90
25. Rawlinson WA (1940) Cristalline hemocyanins: some physical and chemical constants. Austral J Exp Biol Med Sci 18:131–140
26. Rhodes PG, Moser KM (1966) Sources of error in oxygen tension measurement. J Appl Physiol 21:729–734
27. Roughton FJW, Scholander PF (1945) Micro-gasometric estimation of blood gases I. Oxygen. J Biol Chem 148:541–550
28. Scheid P, Kawashiro T (1975) Metabolic changes in avian blood and their effects on determination of blood gases and pH. Respir Physiol 23:291–300
29. Scheid P, Meyer M (1978) Mixing technique for study of oxygenhemoglobin equilibrium: A critical evaluation. J Appl Physiol 45:818–822
30. Severinghaus JW (1968) Measurement of blood gases, p_{O_2} and p_{CO_2}. Ann NY Acad Sci 148: 115–132
31. Severinghaus JW, Bradley BA (1971) Blood gas electrodes or what the instructions didn't say. Radiometer, Copenhagen, ST 59, pp 1–64
32. Sigaard-Andersen O (1974) The acid-base status of the blood. Munksgaard, Copenhagen, pp 175–181
33. Solymar M, Rucklidge MA, Prys-Roberts C (1971) A modified approach to the polarographic measurement of blood O_2 content. J Appl Physiol 30:272–275
34. Sproule BJ, Miller WF, Cushing IE, Chapman CB (1957) An improved polarographic method of measuring oxygen tension in whole blood. J Appl Physiol 11:365–370
35. Tazawa H (1970) Measurement of O_2 content in microliter blood samples. J Appl Physiol 29: 414–416

36. Truchot JP (1978) Variations de la concentration sanguine d'hémocyanine fonctionnelle au cours du cycle d'intermue chez le crabe *Carcinus maenas* (L.). Arch Zool Exp Gen 119:265–282
37. Tucker VA (1967) Method for oxygen content and dissociation curves on microliter blood samples. J Appl Physiol 23:410–414
38. Slyke Van DD, Neill JM (1924) The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. I. J Biol Chem 61:523–573
39. Weber RE, Fänge R, Rasmussen KK (1979) Respiratory significance of priapulid hemerythrin. Mar Biol Lett 1:87–97