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A COMPARISON OF TWO METHODS FOR MEASUREMENT OF O_2 CONTENT OF SMALL (20 μl) SAMPLES OF FISH BLOOD

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Measurements of the oxygen content of blood have been carried out for many years using a variety of methods many of which may be described as volumetric methods, e.g. Van Slyke and Natelson. In recent years methods applicable to small samples (10–20 μl blood) have been developed especially in relation to studies of comparative physiology. Volumetric methods depend upon the release of oxygen chemically bound with haemoglobin, which is frequently achieved by treatment with ferricyanide. With the release of oxygen the resulting increase in P_{O_2} can be measured. If the volume is known then the quantity of oxygen released can be estimated. Methods using this principle have been developed by a number of authors including Fabel & Lübbers (1964) and a method using a small cuvette has been described by Tucker (1967).

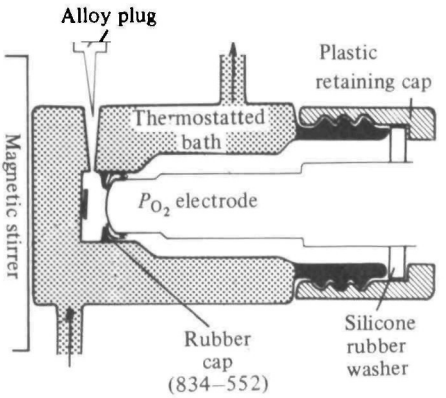
Release of oxygen from a solution can also be obtained by bubbling an oxygen-free mixture that includes carbon monoxide. In a recent commercial instrument (Lex-O_2-con analyser) oxygen is removed from the blood by a scrubber gas before passing over an oxygen-sensitive cell that gives an electrical output the integrated product of which is proportional to the number of oxygen molecules (Amperometric Method). This equipment was originally developed for use with mammalian blood and a number of problems have arisen in relation to its use at the low oxygen contents commonly found in the blood of lower animals. At lower O_2 contents the integrated result tends to be underestimated.

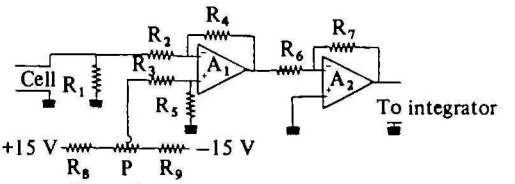
This paper describes a number of modifications which have been made to both standard methods and compares the result obtained for blood taken from the same samples.

Measurements of oxygen content were made either on blood immediately after its removal from an eel or following equilibration with known gas mixtures in the four tonometers of a Radiometer BMS 2 (Mk II) system at 15 °C. Haematocrit value and pH were also determined. For each measurement two samples were taken in rapid succession from the same tonometer, using either a Hamilton syringe for insertion into the Lex-O_2-con apparatus or a disposable micropipette for the Tucker method. Although the measurements were made in the same laboratory, the values obtained were unknown to those operating the respective methods until later.

1. *Volumetric Method*

The method was essentially that described by Tucker (1967) and by Hughes, Palacios & Palomeque (1975). Modifications have arisen because of a change from Beckman to Radiometer (E 5046) oxygen electrodes. It was found desirable to have





P: 10 kΩ (10 t.)
R_2, R_3, R_6 and R_7: 10 kΩ (1%)
R_4, R_5, R_8 and R_9: 100 kΩ (1%)
R_1: 17 Ω (5%)
A_1: A.D. 504 MH analog devices amplifier
A_2: 741 operational amplifier

Fig. 1. Diagrammatic section through cuvette and P_{O_2} electrode used in Volumetric method.
Fig. 2. Circuit diagram of modified electronics used in Amperometric method.

the electrode in a horizontal position and to keep it firmly screwed into the cuvette in order to minimize volume changes in the reaction chamber containing the ferricyanide solution. These modifications are indicated in Fig. 1 from which it is apparent that entrance to the reaction chamber is on the rounded surface of the cuvette and a depression ensures complete filling of the chamber and its entrance channel with ferricyanide solution.

Little trouble has been found with the problem of air bubbles becoming trapped in the new shape of chamber.

A cone-shaped aluminium stopper was used to prevent exchange of oxygen between the ferricyanide and air during a measurement. The distance of the reaction chamber from the outside of the thermostatted cover was minimized (less than 1 cm) in order to ensure effective mixing with the magnetic stirrer. One advantage of the present design is that the stirrer does not come into contact with the electrode membrane.

A rubber cap (Type 834–552) over the electrode and shaping of the thermostatted cuvette ensure a tight fit so that the volume of the reaction chamber did not change during a given series of experiments. Measurement of the chamber volume is critical; the simplest method is to use a finely graduated 1 ml syringe to measure the volume of ferricyanide required to fill the chamber when injected through a needle and thin polyethylene tubing. This procedure was repeated 10 times and the mean (here 0·31 ml) used in the calculations. The stirrers were made from small pieces of Radiometer type D551/S mixing wires covered in polyethylene tubing and sealed at both ends. The mixture used contained 6 g potassium ferricyanide and 3 g Saponin/l. H_2O; deaeration was achieved in a 20 ml syringe by withdrawal of the plunger with its needle inserted in a rubber bung.

2. *Amperometric method*

The amperometric method used in the Lex-O_2-con apparatus was first introduced by Hersch (1952, 1960) and has been discussed in detail by Blayo, Pocidalo & Gaudebout (1978). The main modification was concerned with the electronic network. Current derived from the cell was converted to a voltage and then was fed into an integrator that monitored the total number of oxygen molecules detected by the cell. In the modified apparatus the calibration curves were obtained by injection of various known volumes of distilled water equilibrated with a given gas mixture at a known temper-

Table 1. *Mean values for paired measurements of blood samples and test of significance using paired 't' statistic.*

(A: Equilibrated with gas mixtures of different Po_2; B: blood samples from cannulated Eels.)

	oxygen content (vols %)			
Po_2 (mmHg)	Volumetric	Amperometric	N	Level of significance
(A)				
156·12	9·37	10·97	8	<0·001
10·93	4·92	6·27	6	<0·001
7·83	4·64	5·001	20	<0·001
1·56	0·838	0·814	4	N.S.
		Total for (A)	38	<0·001
(B)				
Arterial blood	4·312	5·176	5	<0·05
Venous blood	2·005	2·31	4	N.S.
		Total for (B)	9	<0·05

Totals of all readings (A+B), $N = 47$, $P < 0{\cdot}001$.

ature. The calibration value for each unit of the integrator is calculated after injection of a known volume of oxygen contained in a sample of 20 μl of air. This calibration curve is a straight line even for injection of small amounts of dissolved oxygen (< 5 nmol).

Measurements of oxygen content of identical samples of blood by the two methods are set out in Table 1. Table 1 A includes results for blood kept in tonometers before the actual sample was taken for analysis. The four tonometers were used simultaneously with the same gas mixture. Some variations were found in the results for different tonometers and consequently part of the variation in the results for a given sample of blood were due to the particular form of tonometry. It is for this reason that standard deviations are not given for the results in Table 1 as this was partly due to the differences in the tonometers as well as variations in the particular technique for measurement of oxygen content. In all cases, a paired t test was applied to results obtained from blood that had been treated in an identical way after removal from the eels. Table 1 B includes results for blood samples obtained from either an artery or vein of the eel and measurements were made immediately following withdrawal from the catheter and were part of routine experiments being carried out in the laboratory.

Out of a total of 47 pairs of measurements, the value obtained with the volumetric method was always lower than that obtained from the Lex-O_2-con with the exception of six cases. In only one of the 47 trials were the two results identical! The paired t statistics indicate quite clearly that there are significant differences between the results obtained with the two methods. There is some suggestion that the differences are less significant for blood equilibrated at lower P_{O_2}'s. In this connexion Adams & Cole (1975) found thet the Lex-O_2-con gives readings reasonably close to predicted values at higher O_2 contents (25–30 vols %).

This comparison has confirmed that both methods are convenient, accurate, and reproducible especially when it is remembered that such small samples are being used. In both cases, errors due to variation in handling by a particular experimenter are important. Once a particular procedure and technique has been developed, the final results are very consistent.

The amperometric method requires a special scrubber gas, whereas this is not required in the Tucker method. In both cases, determinations can be carried out fairly rapidly (Tucker, 7 min total; Lex-O_2-con, 10 min), and because of the less complex arrangement with the volumetric method it is possible to run two cuvettes alternately so that the delay time due to equilibration (4 min) is used for beginning a second determination. Calibration procedures are possibly simpler with the volumetric method once the electrode has been zeroed and calibrated for a particular barometric pressure. Small changes in its calibration are not so serious as the measurement made is a difference between two P_{O_2}'s. This advantage means that different sample sizes can be used without the need to alter any part of the system, but must be taken into account in the calculation. One of the main problems with the operation of the volumetric method is the danger of insertion of small bubbles, but again the operator soon learns to overcome these problems as they arise. The horizontal orientation of the electrode tended to increase some of these problems, but once again the precise way in which the sample is inserted is important. Some of the main disadvantages of the amperometric method are due to its use at low oxygen contents, but modification to the electronics described here make it more sensitive and reliable.

The volumetric method is only available for use with blood measurements whereas the amperometric method may be used not only for gas, water and blood samples, but also for other media. The results obtained with the amperometric method do not depend upon the nature of the medium in which the oxygen is contained. Since the amount of oxygen dissolved from a gas mixture containing oxygen in a given volume of water is theoretically known but not for blood, the Lex-O_2-con is the only one of the two methods which can be completely calibrated.

Other experiments have shown that results with the Lex-O_2-con are not influenced by the volume of the sample (i.e. values obtained for O_2 content with a sample of 200 μl water equilibrated with a gas mixture containing 20% oxygen were identical to those obtained using a 50 μl sample of water equilibrated with a gas mixture containing 80% oxygen). Further verification of the performance of the instrument was obtained using different oxygen contents.

It must be emphasized that for both methods the major errors arise because of inevitable uncertainties in the precise volume injected into the apparatus. The error may amount to about 5% when samples of 10 or 20 μl are used. For both methods the concentration of red cells in the sample may vary in relation to the precise details of the sampling procedure. Therefore it is important in both cases to take precautions to maintain uniformity of the samples that are being used.

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