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IMPORTANT

Both the ferricyanide and the cyanide, which are used in these determinations, are toxic chemicals. As with other potentially hazardous laboratory procedures, responsibility for their

safe use and handling rests with the user. If in doubt, consult your institutional Safety Officer before proceeding.

1. INTRODUCTION

The TC500 Tucker Cell is used to measure the total oxygen content of small volumes of blood, following the method of Tucker (1967) for haemoglobin, and the modification of Bridges et al (1979) for haemocyanin. It is a simple and accurate method and each determination can be completed in less than five minutes.

This instruction manual describes the construction, assembly and volume calibration of the cell (Section 2). In Section 3, a working outline of the procedure is described, but users are recommended to also refer to the original papers cited in the references at the end.

2. CONSTRUCTION AND ASSEMBLY

The Tucker cell comprises a base section into which the electrode is fitted, and a glass water-jacket containing the 500µl sample chamber. When the water jacket is fully screwed onto the base section, the chamber seals against a neoprene gasket, which also makes a seal around the electrode tip and thereby forms the floor of the assembled chamber. The chamber is sealed by a removable acrylic stopper. The stopper has a 1mm diameter central channel, sealed by a 2mm glass ball located in its upper reservoir.

2.1 Assembly

Unscrew the cap at the bottom of the base section of the Tucker cell. Pass this over the cable of the oxygen electrode, which will be already fitted with three 'O'rings. Now insert the electrode into the centre hole and screw the cap full on.

Connect the inlet and outlet tubes of the water jacket to a pumped source of constant temperature water. Allow the water to enter slowly at first, to avoid the production of small air bubbles, which can impair visibility of the sample chamber. Invert the Tucker cell until all air bubbles have been flushed away. Clamp the support rod at a suitable height on a clamp stand, with the Tucker cell in a vertical position.

2.2 Calibration of chamber volume.

With the electrode in position, remove the stopper of the chamber. With fine forceps carefully drop the magnetic stirbar into the base of the chamber. Replace the stopper, pushing it firmly down until its top flange locates on the top of the cell. Introduce distilled water containing a trace of detergent through the channel in the stopper, using a digital autopipette, until the water

just reaches the top of the channel. Alternatively, take a 1ml syringe, fill it with water and detergent, weigh it and inject the water into the chamber, weighing it again when the chamber has been filled to the top of the channel. Convert the weight to volume by assuming that 1g = 1ml.

Remove the stopper, blot it dry on absorbent tissue and carefully suck out the water in the chamber. Final traces of water on the surface of the electrode can be removed by touching the surface with a fine wick of absorbent tissue. This will not damage the membrane of the electrode. Refill the chamber as before and take the mean of three measurements.

It is important that there are no bubbles left in the chamber during this calibration. The detergent in the water will decrease surface tension of the surfaces and hence inhibit bubble formation.

Note the chamber volume. This will not change when the electrode is occasionally removed for membrane replacement.

3. OPERATION

3.1. Blood Oxygen Content Determination.

The method outlined below is based upon the method of Tucker (1967) for haemoglobin-containing blood and a modification of Bridges et al (1979) adaptation for haemocyanin. The operational procedure is outlined below and its theoretical basis is given in the Appendix (Section 5).

Please note: Both ferricyanide and cyanide are toxic chemicals and great care should be exercised at all times. Follow the normal Safety Procedures of your laboratory. The following procedures are provided for guidance only. Each user must, as in any laboratory investigation with toxic chemicals, take responsibility for safe application of these procedures.
The method is described for use with haemoglobin. For haemocyanin-containing blood, use potassium cyanide in place of the potassium ferricyanide solution.

3.1.1. The following items will be required:

Strathkelvin 781,781b or 782 oxygen meters or the 928 interface

Strathkelvin 1302 oxygen electrode

Strathkelvin TC 500 Tucker cell

Magnetic stirrer

Constant temperature water bath set at 32°C +/-0.05°C. *

Aspiration source (for emptying the cell) - preferably a tube terminating in a pasteur pipette and connected to a source of vacuum, such as a water vacuum pump. Alternatively use a pasteur pipette manually.

50m1 syringe (Luer fitting); short hypodermic needle and vinyl tubing. (Needle and tubing are supplied with the TC500)

Hamilton or S.G.E. syringe or similar, with Chaney adaptor, 25µ1 capacity
Potassium ferricyanide solution (for haemoglobin); or potassium cyanide solution (for haemocyanin). Make up fresh each day in a dark glass stoppered reagent bottle and keep at 32°C in a water bath (see Appendix 5.1).

Zero oxygen solution (see Appendix 5.1) made up fresh each day and kept in a 10 or 20m1 syringe, in the waterbath.

Aerated distilled water in a beaker or conical flask, located in the 32°C waterbath and kept aerated with a fine stream of air bubbles.

Optional: Chart recorder, set to a chart speed of 3cm/min. This is not essential but it gives an easy visual check on the progress of the reaction.

* See Section 5.6 number 1

3.1.2.

Fit the 3 way tap to the 50m1 syringe and fit the length of vinyl tubing to the tapered male outlet of the tap. Slide the tubing on until it just adheres; if it is pushed fully on, it will be almost impossible to remove again. With the syringe vertical and pointing downwards, turn the handle of the 3-way tap to the **+** position. Dip the tip of the tubing beneath the level of the ferricyanide in the waterbath and draw in solution until the syringe is about two thirds full. Turn the tap to the **-** position and allow the ferricyanide in the tubing to drain back into the bottle. Carefully slip off the tubing and fit the short hypodermic needle provided. Invert the syringe, turn the tap to the **¦** position and expel air into a paper tissue, which will absorb any solution, which is carried over. Turn the tap to the **-** position thereby sealing the syringe from the outside.

Degas the ferricyanide by carefully and slowly withdrawing the plunger until air bubbles form. Invert the syringe and turn the tap to the **¦** position and again expel the air. Repeat these step 2

or 3 times until no further bubbles form. Ensure that air does not enter the syringe around the plunger during this operation. (If this does happen, try using a new syringe) Now place the syringe in the 32°C waterbath until required.

3.1.3. Calibration of the oxygen electrode:

Calibrate the electrode each day. With the 1302 electrode in place in the cell, and with water at 32°C circulating through the water jacket, remove the stopper and rinse out the cell with distilled water to which a few drops of detergent have been added. Use a pasteur pipette to fill and empty the cell. Take care not to touch the membrane of the electrode, which forms the floor of the cell.

Add zero oxygen solution from the relevant syringe, adding enough to half fill the cell. Refer to the 781, 782 or 928 manual for full calibration instructions. Aspirate the solution from the cell and give several rinses with distilled water. After removing the last distilled water rinse, add the magnetic flea to the cell, and half fill the cell with aerated water from the beaker in the 32°C waterbath using a Pasteur pipette and then replace the stopper. Invert the magnetic stirrer over the top of the stopper, switch on, and stir the water in the cell. Although the magnetic stirrer can be hand-held during this, it may be preferable to secure it in position, when in use, with a laboratory clamp and stand.
Remove the magnetic stirrer; aspirate the excess water from the upper reservoir of the stopper. Remove the stopper and remove the remainder of the water in the cell. Replace the stopper and drop the small glass ball into its upper reservoir.

3.1.4.

Introduce the degassed ferricyanide from the syringe. With the tap in the **+** position, carefully fill the chamber until ferricyanide just overflows past the glass ball into the upper reservoir. Ensure that there are no air bubbles in the chamber. Any bubbles present are most easily seen if a light source is arranged immediately behind the cell. If air bubbles are present, aspirate the liquid from the chamber and repeat this step.

3.1.5.

Replace the magnetic stirrer and switch on. Switch on the chart recorder and wait until the reading stabilises, ideally at an oxygen tension < 100 torr (mm Hg). Note the reading. If the reading is above this, degas the ferricyanide again. Check again to ensure that there are no air bubbles in the chamber.

3.1.6.

Take the blood sample into the Hamilton or S.G.E. syringe and quickly introduce 10µl towards the bottom of the chamber (taking care not to touch the electrode with the needle of the syringe). Replace the magnetic stirrer to mix the contents of the cell. Wait for the meter reading

to stabilise at the higher reading as bound O_2 is released from the haemoglobin into solution, thereby increasing the total PO_2 the solution. The time course of this equilibration is easily followed on the chart recorder. Take a note of the new reading. The difference between this reading and the initial reading is $\triangle PO_2$.

3.1.7.

If another reading is to be taken straight away and if the PO_2 reading on the meter has not risen beyond 110 - 120 torr, a further blood sample may be introduced straight away as in step 3.1.6. Alternatively, rinse out the chamber by adding more degassed ferricyanide through the stopper, aspirating away the previous solution as it is displaced upwards into the upper reservoir. Now return to step 3.1.5.

3.1.8.

Finally rinse out the chamber with distilled water. Remove the stopper, taking care not to lose the glass ball, and rinse in distilled water. Rinse out the Hamilton syringe.

3.2. Calculations.

3.2.1 Vertebrate blood:

For vertebrate blood, which has a high oxygen capacity, the oxygen in physical solution in the plasma is often ignored since it represents a very small proportion of the total. In these cases use the original formula of Tucker (1967) to calculate the bound oxygen concentration:

$$a_f \times cv \times \frac{100}{bv} \times \frac{1}{760} \times \triangle PO_2 \text{ mls } O_2 \text{ blood}$$

Where a_f is the solubility coefficient of ferricyanide or cyanide (Appendix 5.3)
cv is the chamber volume (mls)
bv is the blood sample volume (mls)
$\triangle PO_2$ is the difference between initial and final PO_2 values.

3.2.2 Invertebrate Blood.

In invertebrates, with low oxygen capacity bloods, the dissolved oxygen may be relatively more important. Bridges et al (1979) give methods for the separate calculation of the total oxygen, bound oxygen and physically dissolved oxygen concentrations. The formulae derived from these are given below:

Total oxygen concentration:

$$a_f \times cv \times \frac{100}{bv} \times \frac{1}{760} \left[PO_{2f} - \left(PO_{2i} \times \frac{cv - bv}{cv}\right)\right] \text{mls } O_2 .100\text{mls}^{-1}$$

Bound oxygen concentration:

$$a_f \times cv \times \frac{100}{bv} \times \frac{1}{760} \left[PO_{2f} - \left(PO_{2i} \times \frac{cv - bv}{cv}\right) + \left(PO_{2p} \times \frac{bv}{cv}\right)\right] \text{mls } O_2 .100\text{mls}^{-1}$$

Physically dissolved oxygen concentration:

$$a_p \times cv \times \frac{100}{bv} \times \frac{1}{760} \left[PO_{2p} \times \frac{bv}{cv}\right] \text{mls } O_2 .100 \text{ mls}^{-1}$$

Where a_f is the solubility coefficient of ferricyanide or cyanide (Appendix 5.3)
a_p is the solubility coefficient of plasma (Appendix 5.4)
cv is the chamber volume (mls)
bv is the blood volume (mls)
PO_{2i} is the initial ferricyanide PO_2
PO_{2f} is the final PO_2 after the addition of blood
PO_{2p} is the PO_2 of the plasma (determined beforehand)

The derivation of Tuckers formula is explained in Appendix 5.5.

4. MAINTENANCE

4.1 Electrode removal.

Before removing the electrode to change the membrane, rinse out and aspirate water from the chamber. Unscrew the knurled collar at the base of the electrode holder and withdraw the electrode. It is not necessary to drain the water jacket in order to remove the electrode.

4.2 Cleaning the chamber and water jacket.

The chamber can be cleaned by removing the stopper and rinsing with distilled water. If it becomes necessary to clean the inside of the water jacket, switch off the pumped water supply and drain the water away. Now almost completely unscrew the knurled cap, which holds the

electrode in place to release the tension applied to the electrode by the cap spring. Unscrew the water jacket and wash as required. When reassembling, note that the water jacket and sample chamber seat against their respective seals simultaneously. The water jackets are not interchangeable between different Tucker cell base sections without making an adjustment. If it is necessary to make an interchange, proceed as described in section 4.3.

4.3 Fitting a replacement glass top - P/N SK 500.

Remove the electrode and unscrew the stainless steel support rod from the base section. With the hexagonal 2.5mm 'Allen' key, unscrew the grub screw within and slide the central probe holder downwards by c 1cm. Remove the neoprene gasket (washer) from the tip of the electrode holder. In its place insert the plastic spacer, which is provided with the replacement glass. Screw in the glass until it seals on the bottom 'O' ring. Slide the probe holder upwards until the sample chamber seats against the plastic spacer. Whilst exerting upwards pressure to keep the two surfaces together, use the hexagonal Allen key to retighten the grub screw. Refit the stainless steel support rod. Now unscrew the water jacket, remove the spacer and replace the neoprene gasket. The gasket may appear to be oversize, but this is because the edges of the gasket seating are undercut to hold it in position. Screw in the water jacket again. The sample chamber will now seal against the gasket with the correct amount of compression.

5 APPENDIX.

5.1 Solutions:

1. Zero oxygen solution: Make up 50mls 0.0l M sodium tetraborate ($Na_2B_4O_7$) by dissolving 1.9 g in 500ml distilled water. To this add approximately l0mg sodium sulphite. The concentration of sulphite is not critical, and with practice, the approximate weight can be judged visually on the tip of a spatula. Make up fresh each day.

2. 0.6% Potassium ferricyanide solution. Dissolve 0.6g potassium ferricyanide in l00ml distilled water and keep in a dark glass stoppered bottle. Make fresh each day.

3. 0.6% Potassium cyanide solution. Dissolve 0.6g potassium cyanide in 100ml distilled water and keep in a dark glass stoppered bottle. Make fresh each day.

5.2 Ambient air PO_2:

For calibration of the electrode, with aerated water, ambient air PO_2 is given by:

$$PO_2 = [P_b - P_{w\,v}] \times 0.2095 \text{ torr.}$$

Where P_b = barometric pressure read from a barometer (mm Hg or torr)
P_{wv} = water vapour pressure [=35.66 torr at 32°C]

5.3 Solubility Coefficients a_f for 0.6% potassium ferricyanide:

°C	a_f
25	0.0284
26	0.0279
27	0.0275
28	0.0270
29	0.0265
30	0.0261
31	0.0256
32	0.0252
33	0.0248
34	0.0243

These values are derived from Sendroy et al (1934). If it is necessary to work at temperatures other than these, consult their paper for the relevant value of a.
Bridges et al (1979) make the assumption that the value of a for KCN will be similar to those for potassium ferricyanide.

5.4 Solubility Coefficients a_p for plasma:

In order to determine a_p it is necessary to know the osmolarity of the blood. Seawater has an osmolarity of approximately 1. It is customary to assume that for marine animals, except fish, the plasma corresponds to seawater of 34% salinity. Tables of a for seawater are given by Weiss (1970). Note that the values in his table need to be divided by 100. The values of a have the following approximate range of values:

	Distilled water	**Sea-water Salinity**			
		10‰	20‰	30‰	34‰
5°C	0.0893	0.0836	0.0783	0.0733	0.0714

30°C	0.0510	0.0483	0.0458	0.0434	0.0424

Extrapolation between these values will introduce very little error into the final calculation of the physically dissolved oxygen.

5.5 Principle of the method:

This explains the derivation of the simple formula of Tucker (1967) for use with vertebrate haemoglobins.

1. The oxygen electrode measures the partial pressure of oxygen in solution. The oxygen, which is bound to a respiratory pigment, does not exert a partial pressure. When PO_2 is measured in a blood sample, it is the PO_2 of the oxygen in physical solution in the plasma, which is being detected. If the blood is now treated with an inhibitor such as ferricyanide (for haemoglobin) or cyanide (for haemocyanin), the bound oxygen is liberated and passes into solution in the plasma. The PO_2 of the plasma rises correspondingly. This increase in PO_2 is used to determine the oxygen content and oxygen concentration. In this derivation, the word ferricyanide is used, but the principle is exactly the same with cyanide.

2. Definitions:

   Oxygen concentration is the volume of oxygen in a standard volume of solution and has units such as mls O_2.100mls blood^{-1}

   Oxygen content is the volume of oxygen in a given sample and has units such as mls O_2.

3. The oxygen concentration of ferricyanide solution is given by the solubility coefficient a_f in units of mls O_2.mls ferricyanide^{-1} at 760 torr at a specified temperature. The oxygen concentration in the Tucker Cell initially is therefore

   $$\frac{PO_{2i}}{760} \times a_f \text{ mls } O_2.\text{ml}^{-1}$$

   Where PO_{2i} is the PO_2 of the ferricyanide, read directly from the O_2 meter.

   The initial oxygen content in the cell is given by:

$$\frac{PO_{2i} \times a_f \times cv}{760}$$

Where cv is the chamber volume in mls.

4. Similarly the final oxygen content in the cell, after release of the bound O_2 is

$$\frac{PO_{2f} \times a_f \times cv}{760}$$

Where PO_{2f} is the final reading on the meter.

5. The difference in oxygen content, representing the volume of bound oxygen released is:

$$\left[\frac{PO_{2f}}{760} \times a_f \times cv\right] - \left[\frac{PO_{2i}}{760} \times a_f \times cv\right]$$

or $$\frac{PO_{2f} - PO_{2i}}{760} \times [a_f \times cv]$$

or $$a_f \times cv \times \frac{\triangle PO_2}{760}$$

6. The bound oxygen concentration of the blood sample is therefore

$$a_f \times cv \times \frac{1}{bv} \times \frac{\triangle PO_2}{760} \text{ m1s } O_2.\text{ml blood}^{-1}$$

where bv is the blood sample volume.

7. Hence the bound oxygen concentration in mls O_2 .$100ml^{-1}$ is

$$a_f \times cv \times \frac{100}{bv} \times \frac{\triangle PO_2}{760}$$

5.6 Notes:

1. The methods described by Tucker (1967) and Bridges et al (1979) suggest that the determinations should be carried out at 32°C. At this temperature, release of bound O_2 and equilibration is rapid. However, the method can be used at other temperatures, providing constant temperature is maintained.

2. The volume of blood to be used can be determined empirically. A volume of 5µl can be used with mammalian blood. Volumes of 10µl have given satisfactory results with crustacean haemocyanin-containing blood.

3. It is not essential to use a chart recorder with this method, since readings are taken directly from the meter. However, several users have noted that the chart recording gives a useful visual check of the time course of the equilibration phases.

5.7 References:

Tucker, V. A. (1967) Method for oxygen content and dissociation curves on microlitre blood samples. J. appl. Physiol., 23, 410 - 414.

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